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Context-dependent concordance between physiological divergence and phenotypic selection in  
sister taxa with contrasting phenology and mating systems

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Physiological divergence and phenotypic selection in *Clarkia* sister taxa

38 ABSTRACT

39

40 PREMISE: The study of phenotypic divergence of, and selection on, functional traits in closely  
41 related taxa provides the opportunity to detect the role of natural selection in driving  
42 diversification. When selection in field populations differs between taxa in a pattern that is  
43 consistent with the phenotypic difference between them, this provides evidence that natural  
44 selection reinforces the divergence. Few studies have sought evidence for such concordance for  
45 physiological traits.

46

47 METHODS: Herbarium specimen records were used to detect phenological differences between  
48 sister taxa independent of the effects on flowering time of long-term variation in the climate  
49 across collection sites. In the field, physiological divergence in photosynthetic rate, transpiration  
50 rate, and instantaneous water use efficiency were recorded during vegetative growth and  
51 flowering in 13 field populations of two taxon pairs of *Clarkia*, each comprised of a self-  
52 pollinating and a outcrossing taxon.

53

54 RESULTS: Historically, each selfing taxon flowered earlier than its outcrossing sister taxon,  
55 independent of the effects of local long-term climatic conditions. Sister taxa differed in all focal  
56 traits, but the degree and (in one case) the direction of divergence depended on life stage. In  
57 general, self-pollinating taxa exhibited higher gas exchange rates, consistent with their earlier  
58 maturation. In 6 of 18 comparisons, patterns of selection were concordant with the phenotypic  
59 divergence (or lack thereof) between sister taxa.

60

61 CONCLUSIONS: Patterns of selection on physiological traits measured in heterogeneous  
62 conditions do not reliably reflect divergence between sister taxa, underscoring the need for  
63 replicated studies of the direction of selection within and among taxa.

64

65 Keywords: adaptation, *Clarkia*, divergence, gas exchange, mating system evolution, phenology,  
66 phenotypic selection, water use efficiency, climate.

67

68           The study of phenotypic divergence between, and phenotypic selection within, closely  
69 related taxa provides the opportunity to detect the processes contributing to evolutionary  
70 diversification and trait evolution (Hall and Willis, 2006; Anderson and Gezon, 2015; Teixido et  
71 al., 2019; Custer et al., 2021). Phenotypic divergence may be generated by adaptive evolutionary  
72 responses to short-term or sustained differences between populations in the direction of natural  
73 selection, but also by plastic responses of populations to temporary or longstanding  
74 environmental conditions (which may or may not be adaptive) (Lucek et al., 2014; Frank et al.,  
75 2017; Rajkov et al., 2018). In plants, evidence for both mechanisms (adaptive evolution and  
76 plasticity) have been detected (Eckhart et al., 2004; Whittall and Hodges, 2007; Mazer et al.,  
77 2009, 2010, 2018; Anderson et al., 2012, 2015; Dudley et al., 2012; Briscoe Runquist and  
78 Moeller, 2014; Pilote and Donovan, 2016; Brancalion et al., 2018; Ricote et al., 2019).

79           The current study evaluates the hypothesis that physiological divergence between sister  
80 taxa in the wildflower genus *Clarkia* is consistent with taxon-specific phenotypic selection under  
81 field conditions (cf. Hall and Willis, 2006; Dudley et al., 2012; Anderson et al., 2015). For  
82 example, when closely related sister taxa differ in a functional trait such as leaf size, evidence  
83 that natural selection contributes to this divergence would be either stronger directional selection  
84 favoring larger-leaved individuals in populations of the larger-leaved taxon than in the smaller-  
85 leaved one, or a qualitative difference between taxa in the sign of the selection coefficient for  
86 leaf area, with the larger-leaved taxon experiencing directional selection favoring larger-leaved  
87 individuals, and the opposite pattern applying to the smaller-leaved taxon.

88           Studies examining both divergence between taxa and taxon-specific patterns of selection  
89 are rare, particularly for physiological traits (but see Dudley et al., 2012; Anderson et al., 2015),  
90 in part because characterizing the strength and direction of selection on any trait in wild species

91 requires observations of both functional traits and lifetime fitness in many individuals (Swenson  
92 et al., 2020) and, ideally, in multiple populations. In addition, the phenotype of physiological  
93 traits can be both highly dynamic during development and strongly influenced by environmental  
94 conditions (Ackerly et al., 2000; Caruso et al., 2020), variation in which is difficult to control in  
95 wild populations. Measuring the phenotype of physiological traits should therefore take such  
96 dynamism into account by recording the phenotype of individuals multiple times during their life  
97 cycle and/or by measuring physiological attributes that reflect an individual's lifetime  
98 performance, such as integrated water use efficiency. In the current study, to assess whether  
99 physiological divergence between sister taxa is both detectable and consistently reinforced (or  
100 caused) by phenotypic selection across a heterogeneous landscape, we measured gas exchange  
101 traits and estimated reproductive fitness in many individuals in multiple populations of two pairs  
102 of sister taxa surveyed during both vegetative growth and flowering.

103       Even when taxa differ in the mean values of, and the patterns of selection on, fitness-  
104 related traits, it can be difficult to identify the factors directly responsible for such differences.  
105 Taxa may differ not only in the abiotic conditions and in the composition of co-occurring species  
106 (including competitors, pollinators, pathogens, and herbivores) to which their populations are  
107 exposed, but also in a suite of ancillary (but unstudied) traits that may influence the pattern of  
108 selection on targeted traits. For example, if regionally co-occurring sister taxa differ in flowering  
109 phenology and their populations experience intensifying drought as the flowering season  
110 progresses, then flowering time may affect the rate of water loss through petals due to seasonal  
111 increases in vapor pressure deficit (Teixido et al., 2019). The degree of water loss through  
112 flowers, in turn, may influence the strength of phenotypic selection on water use efficiency  
113 (WUE) exhibited by leaves, with higher WUE being favored in populations or taxa that are under

114 the greatest drought stress during flowering. In this case, considering the flowering phenology of  
115 the focal taxa may help to identify the mechanism responsible for divergence between them in  
116 mean WUE, and to understand the effects of WUE or transpiration rates on individual fitness in  
117 each taxon. Similarly, sister taxa may differ in mating system, with highly autogamous taxa  
118 producing smaller and shorter-lived flowers than their pollinator-dependent sister taxa (Snell and  
119 Aarssen, 2005; Dudley et al., 2007). This difference in flower size and longevity may affect the  
120 strength and direction of natural selection on gas exchange traits, as higher water loss through the  
121 large and longer-lived flowers of outcrossers may drive selection for higher WUE in leaves. In  
122 sum, knowledge of differences between taxa with respect to traits other than those being directly  
123 examined can inform our understanding of the factors that drive the differences observed in the  
124 focal traits.

125         Here, we use a combination of historical herbarium records and contemporary studies of  
126 phenotypic selection and physiological performance to evaluate the ecological factors and  
127 evolutionary mechanisms that have led to physiological differences, expressed in wild populations,  
128 between congeneric sister taxa that differ in both flowering time and mating system. The genus  
129 *Clarkia* is a model system that has been the focus of several decades of research to detect adaptive  
130 differentiation between taxa in habitat preferences and in morphological, life history, reproductive,  
131 and physiological traits. Two pairs of closely related taxa with contrasting mating systems — the  
132 predominantly outcrossing *C. unguiculata* and the highly autogamous *C. exilis*, and the pollinator-  
133 dependent *C. xantiana* ssp. *xantiana* and its selfing sister taxon, *C. xantiana* ssp. *parviflora* —  
134 have been studied most intensively. In each taxon pair, self-pollinating and outcrossing taxa differ  
135 in their geographic distributions and microhabitat preferences (Lewis and Lewis, 1955; Vasek,  
136 1968; Vasek and Sauer, 1971; Eckhart and Geber, 1999). Under experimental conditions, the self-

137 pollinating taxa also flower earlier or produce sequential flowers in more rapid succession than  
138 their outcrossing counterparts (Dudley et al., 2007). In addition, each self-pollinating taxon  
139 produces smaller flowers with lower pollen:ovule ratios, and smaller leaves and seeds than its  
140 outcrossing sister taxon (Runions and Geber, 2000; Delesalle et al., 2008). The selfers have also  
141 been found to exhibit higher gas exchange rates during vegetative growth and/or during flowering  
142 (Mazer et al., 2010; Dudley et al., 2012). In both taxon pairs, divergence between taxa in  
143 vegetative, physiological, or phenological traits is in some cases consistent with the pattern of  
144 selection in these traits (Dudley et al., 2012; Anderson et al., 2015). Due to the dynamic nature of  
145 plant physiological traits over time and space (Ackerly et al., 2000), a more comprehensive  
146 assessment of the degree to which physiological divergence and selection is concordant is needed.

147         In the current study, we first used herbarium-derived data to evaluate whether differences  
148 in mating system between the focal *Clarkia* sister taxa are associated with intrinsic differences  
149 between them in flowering time, independent of the chronic climatic conditions to which  
150 populations are exposed. Previous studies of these taxa have reported that the selfing taxa (*C.*  
151 *exilis* and *C. xantiana* ssp. *parviflora*) flower earlier than their outcrossing sister taxa (Lewis and  
152 Lewis, 1955; Dudley et al., 2007; Anderson et al., 2015), but this is the first study to control for  
153 geographic variation in long-term climatic conditions across each taxon's range when comparing  
154 selfing and outcrossing sister taxa with respect to their flowering phenology. For these  
155 phenological comparisons, we analyzed hundreds of georeferenced herbarium specimen records  
156 of individuals collected across each taxon's geographic range to measure phenological divergence  
157 between sister taxa.

158         We then used physiological observations recorded in spring 2010 for >1800 individuals  
159 in 13 field populations to determine whether sister taxa with contrasting mating systems differ

160 consistently throughout the growing season with respect to gas exchange rates and instantaneous  
161 WUE ( $WUE_i$ ). Based on previous observations of the relative flowering times of our focal taxa  
162 in the Sierra Nevada foothills of California, along with the seasonal drought that occurs in this  
163 region each year as spring progresses, we hypothesized that, during flowering, the earlier-  
164 flowering selfing taxa would generally be exposed to lower dehydration risk than their  
165 outcrossing sister taxa. This ecological difference between sister taxa should result either in the  
166 evolution of higher mean photosynthetic and transpiration rates and lower water use efficiency in  
167 populations of the selfing taxa relative to their outcrossing counterparts or in taxon-specific  
168 plastic responses that generate the same pattern of divergence. To determine whether patterns of  
169 phenotypic selection on physiological traits during vegetative growth and/or flowering are  
170 consistent with the divergence between taxa, we then conducted selection gradient analyses to  
171 estimate direct and total selection on each focal trait within each life stage and taxon. Finally, the  
172 patterns of selection and physiological divergence detected in the current study were compared  
173 to those observed in 2008, when a similar study was conducted in the same region (Mazer et al.,  
174 2010). The high lability of both divergence and patterns of selection reveal the importance of  
175 replicating observations among years, populations, and sampling periods. In addition, examining  
176 the combination of phenological and physiological divergence between taxa facilitated the  
177 interpretation of the patterns observed.

## 178 **MATERIALS AND METHODS**

179 *Study species*—*Clarkia unguiculata* Lindley and *C. xantiana ssp. xantiana* A. Gray are  
180 self-compatible winter annuals that flower in late spring, are endemic to California, and occupy  
181 oak and pine woodlands, grasslands, and roadside habitats, California. While *C. xantiana ssp.*  
182 *xantiana* is restricted to the southern Sierra Nevada and Transverse ranges to the south, the

183 geographic range of *C. unguiculata* is much more widespread, occupying both the Sierra Nevada  
184 and the Coastal and Transverse Ranges (Lewis and Lewis, 1955). In the Lake Isabella region of  
185 the southern Sierra Nevada (Kern and Tulare counties, California, USA), both taxa may occur  
186 alone or in sympatry with their predominantly selfing sister taxa (*C. exilis* and *C. xantiana* ssp.  
187 *parviflora*, respectively; Fig. 1). Where sister taxa co-occur, reproductive isolation is reinforced  
188 by differences between them in flowering phenology and floral morphology, with selfers  
189 flowering earlier and showing lower levels of herkogamy and dichogamy than their bee-  
190 pollinated outcrossing counterparts (Eckhart and Geber, 1999; Dudley et al., 2007).

191 In the southern Sierra Nevada, the sister taxa studied here differ with respect to habitat.  
192 *C. exilis* is sometimes sympatric with *C. unguiculata*, but the latter occupies a broader range of  
193 habitats, growing in woodlands and encroaching into grasslands. By contrast, *C. exilis* is  
194 restricted to low-elevation woodlands and has a narrower, more patchy distribution than *C.*  
195 *unguiculata*. Where the species co-occur, *C. exilis* is found near streambeds, boulders, or rocky  
196 outcrops that provide some shade and/or that retain more soil moisture (Vasek, 1958). The joint  
197 distributions of *C. xantiana* ssp. *xantiana* and ssp. *parviflora* follow a west to east gradient, with  
198 *xantiana* occurring in populations to the west and *parviflora* occurring towards the east, nearly  
199 reaching the Mojave desert. Although there is a narrow central zone of contact between these  
200 subspecies (Eckhart et al., 2004; Geber and Eckhart, 2005), sites occupied solely by ssp.  
201 *parviflora* receive less precipitation than those occupied solely by ssp. *xantiana*.

202  
203 ***Historical climatic and phenological differences between sister taxa: herbarium-based***  
204 ***study***—Occurrence data and the GPS coordinates of all specimen records available for each of the  
205 focal taxa were downloaded from the Consortium of California Herbarium portal (<https://cch2.org>)  
206 on July 31, 2021 (Fig. 2). The date of specimen collection was converted to day-of-year (DOY: 1-  
207 365; 366 for leap years). The GPS coordinates of each collection site were then used to extract

208 from the climate database, ClimateNA v.7.01 (Wang et al., 2016), the mean values of 24 annual  
209 climatic variables estimated from 1951-1980, the 30-year period preceding the point at which  
210 climate warming is considered to have begun to accelerate (IPCC, 2013). The climate variables  
211 estimated are defined in Appendix S1 (see Supplementary Data with this article). To estimate  
212 climatic parameters, ClimateNA uses interpolated values from the PRISM climate database  
213 (PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>) to generate scale-  
214 free estimates of local climatic conditions at a resolution of 4km x 4km.

215         The DOY of specimen collection does not provide a precise record of the date of first  
216 flower or of peak flowering of a specimen because an individual plant collected in flower may  
217 have been collected at any time after flowering has begun, and the duration of an individual's  
218 flowering period may be several weeks or longer. Nevertheless, the date of specimen collection  
219 has been found to be a reliable proxy for flowering time (Davis et al., 2015), and has been used in  
220 many studies to detect the factors influencing flowering phenology (Davis et al., 2015; Matthews  
221 and Mazer, 2016; Willis et al., 2017; Park and Mazer, 2018, 2019; Ellwood et al., 2019; Love et  
222 al., 2019; Park et al., 2019; Mazer et al., 2021; Park, Breckheimer, et al., 2021; Park, Ramirez-  
223 Parada, et al., 2021; Pearson et al., 2021). Among the focal taxa of this study, herbarium  
224 specimens with images that were available for downloading on 25 December 2021 from the  
225 Consortium of California Herbaria website (<https://www.cch2.org>) comprised 26, 679, 10, and 33  
226 specimens of *C. exilis*, *C. unguiculata*, *C. xantiana* ssp. *parviflora*, and *C. xantiana* ssp. *xantiana*,  
227 respectively. Each image was examined to determine whether the individual plant(s) on each  
228 sheet bore one or more open flowers. Of these, 24 (92%), 659 (97%), 8 (80%), and 30 (91%)  
229 specimens bore one or more open flowers, indicating that the DOY of specimen collection for  
230 these taxa generally reflects the specimen's flowering time at a given site.

231  
232        *Statistical analysis of herbarium-derived data and climate*—Closely related taxa might  
233 differ with respect to mean flowering time due to evolutionary and/or short-term plastic  
234 responses to climate. To test for phenological differences between sister taxa that are  
235 independent of variation in local climatic conditions, we controlled statistically for climatic  
236 variation among collection sites when testing for differences between taxa in mean flowering  
237 time. To characterize each collection site using a multivariate index of its climate, principal  
238 components (PCs) for the 24 annual climatic variables were estimated using the pooled data for  
239 each pair of sister taxa. In both pairs of taxa, the first two principal components (PC1 and PC2)  
240 for the 24 climatic parameters together account for >75% of the variance in climate (see  
241 Appendices S2 and S3 for contributions and loadings, respectively).

242        For each data set, the first two PCs were used to determine whether sister taxa differ with  
243 respect to their flowering phenology (controlling for site-specific climatic conditions) and in the  
244 chronic climatic conditions to which they are exposed. The function *prcomp* was used to estimate  
245 PCs, following centering and scaling of the data to unit variance; the function *fviz\_pca\_var* in the  
246 package *factoextra* (v.1.0.7, Kassambara and Mundt, 2020) was used to visualize the  
247 contributions of each variable in each PC. These and all subsequent analyses were conducted in  
248 RStudio (v. 1.4.1717) with R v. 4.1.1.

249  
250        *Phenological differences between sister taxa*—To determine whether sister taxa differ in  
251 their mean flowering date while controlling for the effects of geographic variation in local  
252 chronic climatic conditions (which influence DOY; see Results), we conducted four linear  
253 regressions on each sister pair (Table 1). In all of these models, the day of year of specimen

254 collection (DOY) was the response variable. In neither taxon pair were either of the two-way  
255 interactions (Taxon x PC1 or Taxon x PC2) statistically significant. Below, we present the results  
256 of the model with the lowest AIC value. Type III sums of squares were used to detect a  
257 significant difference between sister taxa in their mean date of collection, controlling for  
258 variation among collection sites with respect to the covariates. The *lm* function with the default  
259 setting for contrasts and the *lme4* package (v. 1.1-27.1, Bates et al., 2015) were used.

260  
261 *Climatic differences between sister taxa*—To determine whether sister taxa differ with  
262 respect to the chronic climatic conditions at their respective herbarium specimen collection sites, we  
263 used linear models to detect statistically significant differences between taxa with respect to the  
264 mean values of the first and the second PCs. In these analyses, either PC1 or PC2 was the response  
265 variable, and taxon was the predictor variable. The *lm* function (using the default setting for  
266 contrasts: `c[contr.treatment, contr.poly]`) in the *lme4* package (v. 1.1-27.1, Bates et al., 2015) was  
267 used.

268  
269 *Contemporary differences between sister taxa: phenotypic divergence in physiological*  
270 *traits*—*Sampling of extant populations: sample sizes and life stages*—Naturally occurring field  
271 populations were surveyed in the region of Lake Isabella, California, USA, in the southern Sierra  
272 Nevada, where we sampled populations at elevations ranging from 457 to 1628 m. In spring 2010,  
273 13 sites were identified that, at the onset of spring, contained a population of one or more of our  
274 focal taxa with a sufficient number of healthy juvenile plants that we judged we would be able to  
275 survey at least 90 individuals for their physiological performance during both vegetative and  
276 flowering stages (Fig. 3; see Appendices S4 and S5 for a summary of each population's GPS

277 coordinates, elevation, sampling dates, and sample sizes). Each population was surveyed once  
278 during vegetative growth and once during flowering in spring 2010; a different group of individuals  
279 was sampled at each life stage. For each survey, 90 individuals were selected at random positions  
280 along one or more transects traversing the population, with the restriction that a surveyed plant had  
281 to bear at least one leaf long enough to sample with a LiCoR-6400 gas exchange analyzer (leaves  
282 that were too short did not extend into the leaf chamber). Sampled plants were typically at least 1m  
283 apart from one another. In total, we measured the physiological performance of >1800 individuals.  
284 We analyzed and compared these traits while controlling statistically for leaf position on the  
285 primary stem and for leaf temperature at the time of measurement, both of which can influence  
286 measures of physiological performance (Mazer et al., 2010).

287

288 *Physiological survey*—Gas exchange measurements were recorded on leaves produced  
289 by the primary branch on each plant. Measured leaves remained attached to the stem while using  
290 a portable infrared gas exchange analyzer (IRGA; LI-COR 6400, Lincoln, Nebraska, USA). We  
291 recorded photosynthetic rate ( $A$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $E$ ;  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and  
292 the derived parameter,  $A/E$ , instantaneous water use efficiency ( $\text{WUE}_i$ ;  $\mu\text{mol}_{\text{CO}_2} \text{ mol}_{\text{H}_2\text{O}}^{-1} \times$   
293  $0.0001$ ). In addition, the position of each measured leaf, recorded as the number of the node that  
294 bore it, was recorded; the node just above the cotyledons was referred to as node 1, with  
295 subsequently produced nodes counted in sequence. The temperature of the leaf ( $^{\circ}\text{C}$ ) at the time  
296 of measurement was also recorded. Node number and leaf temperature ( $T_{\text{leaf}}$ ) were included as  
297 covariates in our statistical models in order to control for their potential effects on physiological  
298 rates, as observed by Mazer et al. (2010). The following LI-COR settings were used: light  
299 source, 6400-40 fluorometer; stability, values of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  remained stable for at least 15 s

300 with a change in slope of  $<1$ ; stomatal ratio = 1; flow =  $500 \mu\text{mol s}^{-1}$ ;  $\text{PAR}_i$  (PAR in the leaf  
301 chamber) =  $1500 \text{ mol m}^{-2} \text{ s}^{-1}$ .  $\text{CO}_2$  concentration in the IRGA reference chamber was 400 ppm.

302 For leaves too narrow to fill the entire  $2\text{cm}^2$  circular area exposed within the LI-COR  
303 6400's leaf chamber, the actual leaf area exposed was calculated and then the LI-COR 6400's  
304 Simulator Software was used to adjust those physiological parameters that were influenced by  
305 leaf area. To measure the leaf area exposed within the chamber, we used an ink pad to apply  
306 black ink to the foam gasket of the chamber; when the chamber was then closed upon the leaf,  
307 the gasket stamped with ink the outer boundary of the leaf's area that was exposed within the  
308 chamber. Following gas exchange measurements, each leaf that failed to fill the chamber's  
309 circular opening was removed, taped to a piece of paper with transparent tape, and subsequently  
310 scanned using a digital scanner. We then used the image analysis program ImageJ  
311 (<http://rsb.info.nih.gov/ij/index.html>) to measure the area of each leaf that had been exposed  
312 within the LI-COR chamber during gas exchange measurement.

313 Instantaneous gas exchange measurements and  $\text{WUE}_i$  provide point estimates of the  
314 physiological performance of individual plants and are not equal to the same parameters  
315 measured over the entire lifespan of individuals (Zelitch, 1982; Serichol-Escobar et al., 2016). In  
316 the current study, however, the large sample sizes obtained per taxon, the replication of each  
317 taxon within and among 2-4 populations, and the statistical control of variation in other plant  
318 attributes that are correlated with instantaneous gas exchange rates (e.g., leaf position on the  
319 stem, leaf temperature at the time of measurement, and the life cycle stage at which plants are  
320 measured) helped to account for the variance in physiological performance that can obscure  
321 differences between taxa. Numerous other recent studies have compared taxa, experimental  
322 treatments, or life history stages based on instantaneous gas exchange measurements (Mazer et

323 al., 2010; Franks, 2011; Dudley et al., 2012, 2015; Gorai et al., 2015; Cheng et al., 2017; Gulias  
324 et al., 2018; Velikova et al., 2018; Wang et al., 2019; Scaloni et al., 2021).

325 *Fitness estimation*—In late June to mid-July 2010, all surviving plants were surveyed  
326 and, when flower production had ceased, the total number of full-sized or mature fruits borne by  
327 each plant was counted to provide an estimate of lifetime reproductive fitness. Many plants,  
328 however, experienced high fruit predation by the larvae of *Hyles lineata* (the white-lined sphinx  
329 moth: Sphingidae) and, at some locations, by vertebrates, so total fruit production could not be  
330 accurately measured on these individuals. Consequently, as plants fully senesced and dried, we  
331 collected all above-ground parts (including fruits) from each plant, air-dried each skeleton in a  
332 paper bag, and returned them to the lab for weighing. All fruits retained by each plant were  
333 removed and counted, and the above-ground stem biomass was recorded (in mg, without any  
334 leaves, flowers, or ovaries). To assess whether above-ground stem biomass is a reasonable proxy  
335 for lifetime reproductive success in these *Clarkia* taxa, we measured the relationship between  
336 above-ground stem biomass and total fruit production among individuals in each taxon that were  
337 sampled during flowering and that experienced no fruit predation. In all taxa, the correlation  
338 coefficient between these log-transformed variables was  $> 0.60$  (*C. unguiculata*,  $r = 0.61$ ,  $n =$   
339  $151$ ,  $p < 0.0001$ ; *C. exilis*,  $r = 0.90$ ,  $n = 60$ ,  $p < 0.0001$ ; *C. xantiana* ssp. *xantiana*,  $r = 0.76$ ,  $n =$   
340  $173$ ,  $p < 0.0001$ ; *C. xantiana* ssp. *parviflora*,  $r = 0.84$ ,  $n = 81$ ,  $p < 0.0001$ ).

341 In the analyses of phenotypic selection described below, for all individuals, we used  
342 above-ground stem biomass as the response variable (i.e., the estimate of individual fitness) (cf.  
343 Donovan et al., 2009). The use of final stem biomass as a proxy for fitness resulted in inflated  
344 fitness estimates for heavily attacked plants (relative to their actual fitness). However, fruit

345 predation occurred late in an individual's reproductive cycle, when the vast majority of its fruits  
346 were developing or fully developed, and new flowers were no longer being produced. Therefore,  
347 the final above-ground stem biomass of a harvested individual was not strongly or directly  
348 influenced by its predation status, and we assume that the stem biomass of a heavily attacked  
349 individual is a good estimate of its potential fitness in the absence of herbivory.

350 *Climate data* — To assess the influence of recent climatic conditions on the physiological  
351 rates of populations and taxa, monthly climatic data (cumulative precipitation; mean minimum,  
352 maximum and mean temperature; and vapor pressure deficit [VPD]) was extracted from the  
353 PRISM climate database (PRISM Climate Group, Oregon State University,  
354 <http://prism.oregonstate.edu>) for November 2007-June 2008 (corresponding to the growing season  
355 surveyed by Mazer et al., 2010) and for November 2009-June 2010 (the growing season of the  
356 current study). These data were examined to determine whether differences between years in the  
357 magnitude or direction of physiological divergence between sister taxa was associated with year-  
358 specific climate. In addition, these climate data were used to examine whether physiological  
359 differences between sister taxa observed during flowering in the current study were associated with  
360 the mean climatic conditions to which their sampled populations were exposed in the month when  
361 they were surveyed (Tables S3, S4). When comparing *C. exilis* and *C. unguiculata*, climatic  
362 conditions for the months of April and May 2010, respectively, were used; these corresponded  
363 most closely to the months when each species were surveyed. When comparing the subspecies of  
364 *C. xantiana*, climatic conditions for May 2010 were used for ssp. *xantiana*, except for the  
365 Democrat Springs and Saw Mill Road sites, for which conditions in June 2010 were used due to  
366 their mid-June survey dates (Appendix S6). For *C. xantiana* ssp. *parviflora*, climatic conditions

367 for May 2010 were used, except for the Long Valley population, for which June 2010 conditions  
368 were used.

369  
370 *Statistical analyses of field data—Physiological divergence between sister taxa—Model*  
371 structure and parameter estimation—To assess phenotypic divergence, we considered 12  
372 separate linear mixed-effects models, accounting for three gas exchange response variables  
373 (photosynthesis, transpiration, and WUE<sub>i</sub>) in both vegetative and flowering stages, for each pair  
374 of sister taxa (3 traits x 2 stages x 2 taxon pairs). The 12 models are all of the form:

375 
$$\text{GasExchangeTrait} \sim \text{Tleaf} + \text{Node Number} + \text{Taxon} + (1 \mid \text{Population})$$

376 where GasExchangeTrait is either photosynthesis, transpiration, or WUE<sub>i</sub>, and Population is  
377 treated as a random effect nested within Taxon. Due to non-normality and heterogeneity in the  
378 residuals, we used bootstrap analyses to compute confidence intervals (CI) for the differences  
379 between sister taxa in physiological traits, and Bonferroni corrections were applied. Where the  
380 95% CI for the Taxon effect does not overlap zero, this is interpreted as a significant difference  
381 between taxon means.

382

383 *Differences between sister taxa in phenotypic selection—Model structure and parameter*  
384 estimation—We assessed the strength and direction of phenotypic selection on each  
385 physiological trait, estimating both total selection (where each physiological trait was used as the  
386 sole predictor variable in its own model) and direct selection, in which both photosynthesis and  
387 transpiration were included as predictor variables in the same model in order to detect the  
388 independent effects of each of these traits on individual fitness.

389 The phenotypic selection analyses tested five distinct models for each taxon pair and  
390 growth stage:

391 Model A: Total selection on photosynthetic rate (P):  
 392 Biomass ~ P + Taxon + Node Number + Tleaf + Taxon x P + (1|Population);  
 393 Model B: Total selection on transpiration (T):  
 394 Biomass ~ T + Taxon + Node Number + Tleaf + Taxon x T + (1|Population);  
 395 Model C: Total selection on WUE:  
 396 Biomass ~ WUE + Taxon + Node Number + Tleaf + Taxon x WUE + (1|Population);  
 397 Model D: Direct selection on photosynthetic and transpiration rates:  
 398 Biomass ~ P + T + Taxon + Node Number + Tleaf + Taxon x P + Taxon x T + (1|Population);  
 399 Model E: Direct selection:  
 400 Biomass ~ P + T + Taxon + Node Number + Tleaf + Taxon x P + Taxon x T + P x T + (1 |  
 401 Population).

402 Model E included the P x T interaction to determine whether the effects on individual  
 403 fitness of photosynthetic or transpiration rates are independent rather than being influenced by  
 404 the value of the other trait. Quadratic terms are not included in these models because they were  
 405 always non-significant ( $\alpha = 0.05$ ).

406 In each of the random intercept models analyzed here, we regarded population as a  
 407 random effect that is nested within taxon, so we adopted a variance structure allowing different  
 408 residual variances for each taxon. See, for example (Zuur et al., 2009; Chapter 19) and the R  
 409 code provided in the supplementary material. These models also account for the effects of leaf  
 410 position and temperature by including them as covariates.

411 Because the number of populations sampled per taxon was relatively small and the  
 412 residual patterns for most of these models exhibited non-normality and heterogeneity of  
 413 variance, the conditions for maximum likelihood or restricted maximum likelihood methods of

414 parameter estimation were violated. In this situation, a more robust estimation procedure is to use  
415 nonparametric resampling. Specifically, we used the *cases bootstrap* method, which resamples  
416 the individuals within population clusters (Leeden et al., 2008). Each confidence interval was  
417 computed from 20,000 bootstrap samples using the *lmeresampler* package (Loy et al., 2021).

418

419 ***Concordance vs. discordance between phenotypic divergence and phenotypic***

420 ***selection***—We examined patterns of phenotypic divergence between sister taxa and the direction  
421 and magnitude of taxon-specific phenotypic selection on each trait during vegetative growth and  
422 during flowering to determine whether the direction and statistical significance of phenotypic  
423 divergence is consistent with the difference between taxa in the direction or strength of selection.  
424 For each trait, we determined whether there is concordance between: the taxon-specific patterns  
425 of selection vs. phenotypic divergence during vegetative growth; the taxon-specific patterns of  
426 selection vs. phenotypic divergence during flowering; and the patterns of selection during  
427 vegetative growth vs. phenotypic divergence between sister taxa expressed during flowering. We  
428 were interested in the latter comparison because differences between sister taxa in the intensity  
429 or direction of selection during vegetative growth could generate phenotypic divergence between  
430 them later in the life cycle. For example, if during vegetative growth selection favored rapid  
431 transpiration rates in *C. exilis* (but was neutral in *C. unguiculata*) resulting in disproportionately  
432 high survivorship of *C. exilis* individuals with high transpiration rates, this could lead to higher  
433 mean transpiration rates in *C. exilis* than in *C. unguiculata* during flowering. In this case, taxon-  
434 specific patterns of selection early in the life cycle would be interpreted as causing phenotypic  
435 divergence between sister taxa later in the life cycle.

436 Concordance is identified where: (a) there is no difference between sister taxa in mean  
437 phenotype or in the magnitude of the selection gradients (i.e., there is no Taxon x Trait

438 interaction affecting total biomass) or (b) sister taxa differ in mean phenotype, and the direction  
439 of selection on the focal trait differs between sister taxa in a manner that is consistent with the  
440 phenotypic divergence between them. Discordance is identified where: (a) there is no difference  
441 between sister taxa in mean phenotype, but the direction of selection differs qualitatively  
442 between them (i.e., there is a significant Taxon x Trait interaction), or (b) sister taxa differ  
443 significantly in mean phenotype, but the direction of selection on the focal trait does not differ  
444 between them (i.e., there is no Taxon x Trait interaction), or (c) sister taxa differ in mean  
445 phenotype, but the direction of selection on the focal trait differs between them in way that is  
446 inconsistent with the phenotypic divergence between them (Table 2).

447

## 448 **RESULTS**

449

### 450 **Historical phenological and climate differences between sister taxa: herbarium-based**

451 **study**—*Phenological divergence between sister taxa* — Of the four linear models tested (Table 1)  
452 to detect differences between sister taxa in the DOY of specimen collection (controlling for  
453 variation in PC1 and PC2), the model with the lowest AIC value was Model 2 for *C. unguiculata*  
454 and *C. exilis*, and Model 3 for *C. xantiana* ssp. *xantiana* and ssp. *parviflora* (Table 1). In both pairs  
455 of sister taxa, the selfing taxon was collected in flower earlier than the outcrossing taxon (Table 3).  
456 *C. exilis* was collected on average 11 days earlier than *C. unguiculata*, and *C. xantiana* ssp.  
457 *parviflora* was collected on average six days earlier than *C. xantiana* ssp. *xantiana* (Fig. 4).

458

459 *Climatic differences between sister taxa* —In the pooled data for *C. unguiculata* and *C.*  
460 *exilis*, PC1 and PC2 account for 46% and 31% of the variance in climate, respectively; in *Clarkia*  
461 *xantiana* ssp. *xantiana* and ssp. *parviflora*, PC1 and PC2 explain 62% and 21% of the variance,  
462 respectively (Appendix S2). Across the geographic distributions of both taxon pairs, the loadings

463 of PC1 indicate that it is an axis dominated by temperature, while PC2 is an axis dominated by  
464 humidity (Appendix S3).

465 In each taxon pair, sister taxa differ with respect to long-term (1951-1980) mean climatic  
466 conditions at the sites of specimen collection (Table 4; Fig. 5). The analysis of the pooled data  
467 for *C. unguiculata* and *C. exilis* detected a significant difference between them in the mean value  
468 of PC2 (but not PC1), with *C. exilis* exhibiting a lower mean than *unguiculata*. On average, *C.*  
469 *exilis* occupies warmer and drier sites than *C. unguiculata*; sites with *C. exilis* are associated with  
470 lower relative humidity (RH), a higher mean Hargreaves cumulative moisture deficit (CMD),  
471 lower cumulative moisture index (CMI), higher evaporation, and a higher mean annual  
472 temperature (MAT) than sites with *C. unguiculata* (Appendix S3). This difference is partly due  
473 to *C. unguiculata*'s larger geographic range (Fig. 2), particularly its occupation of sites at higher  
474 elevations and in more northern and coastal regions.

475 The analysis of the pooled data for *Clarkia xantiana* ssp. *xantiana* and ssp. *parviflora*  
476 detected a significant difference between them with respect to the PC1 (but not PC2), with ssp.  
477 *parviflora* exhibiting a higher mean value than ssp. *xantiana* (Table 4). Within its geographic  
478 range, the herbarium records suggest that *C. xantiana* ssp. *parviflora* occupies sites that were  
479 historically (from 1951-1980) cooler than those occupied by ssp. *xantiana*. The sites from which  
480 ssp. *parviflora* was collected are associated with higher CMI, more degree days < 18°C, more  
481 degree days < 0°C, fewer degree days > 5°C, and lower MAT than those from which ssp.  
482 *xantiana* was collected (Appendix S3 and Fig. 5).

483  
484 ***Contemporary differences between sister taxa: phenotypic divergence***—In both taxon pairs, the  
485 sister taxa differ with respect to all three physiological traits, but the magnitude and the direction  
486 of the divergence differed between growth stages (Table 5; Fig. 6; see Appendix S7 for

487 application of Bonferroni correction). *C. exilis* and *C. unguiculata* did not differ physiologically  
488 during vegetative growth, but during flowering, *C. exilis* exhibited higher photosynthetic and  
489 transpiration rates, and lower WUE<sub>i</sub>, than *C. unguiculata*. *Clarkia xantiana* ssp. *parviflora*  
490 differed from *C. xantiana* ssp. *xantiana* during both growth stages, but the traits and the direction  
491 of the difference changed over time. During vegetative growth, *C. xantiana* ssp. *parviflora*  
492 exhibited a higher transpiration rate and lower WUE<sub>i</sub> than *C. xantiana* ssp. *xantiana*, while  
493 during flowering ssp. *parviflora* exhibited a lower mean photosynthetic rate and higher WUE<sub>i</sub>  
494 than ssp. *xantiana*.

495

496 ***Contemporary differences between sister taxa: phenotypic selection***—Differences  
497 between sister taxa in the strength or direction of phenotypic selection on the three focal traits  
498 were common in *C. unguiculata* and *C. exilis*, but undetectable in *C. xantiana* ssp. *xantiana* and  
499 ssp. *parviflora* (Fig. 7). During both growth stages, significant Taxon x Trait interactions were  
500 detected for transpiration and WUE<sub>i</sub> in *C. unguiculata* and *C. exilis* (Table 6). During vegetative  
501 growth, phenotypic selection favored high transpiration rates and low WUE<sub>i</sub> in *C. exilis* but not  
502 in *C. unguiculata* (at  $p < 0.01$ ; Fig. 7; Table 6). During flowering, selection favored high  
503 transpiration rates in *C. unguiculata* and low transpiration rates in *C. exilis*, and selection favored  
504 high WUE<sub>i</sub> in *C. exilis* but not in *C. unguiculata*. The patterns of direct vs. total selection on  
505 photosynthetic and transpiration rates did not differ (Fig. 7a vs. 7b).

506

507 ***Concordance between phenotypic divergence and phenotypic selection***—All three  
508 ecophysiological traits exhibited context-specific concordance between the phenotypic  
509 divergence between sister taxa and the direction or strength of selection; the degree and type of

510 concordance depended on taxon pair and developmental stage (Table 7). In both taxon pairs, the  
511 patterns of concordance exhibited by direct selection on photosynthesis and transpiration are the  
512 same as those exhibited by total selection, so only the latter are described here.

513         In *C. unguiculata* and *C. exilis*, among the nine comparisons made between physiological  
514 divergence and the taxon-specific directions of total selection on each trait, three cases of  
515 concordance were detected. Photosynthesis exhibited concordance (Type 1, Table 2) during  
516 vegetative growth; the species did not diverge phenotypically nor did their selection gradients  
517 differ (Table 7). In this taxon pair, both transpiration and  $WUE_i$  exhibited concordance (Type 2)  
518 when the phenotypic divergence observed in the flowering stage is compared to the pattern of  
519 selection observed during vegetative growth. In *C. xantiana* ssp. *xantiana* and *C. xantiana* ssp.  
520 *parviflora*, among the nine comparisons between divergence and taxon-specific total selection,  
521 three cases of concordance were detected. Photosynthesis exhibited concordance (Type 1) during  
522 vegetative growth; the sister taxa did not differ phenotypically or in their selection gradients.  
523 Transpiration also exhibited concordance (Type 1) when selection and divergence were  
524 measured during flowering, and when divergence during flowering was compared to taxon-  
525 specific selection during vegetative growth. No cases of concordance were detected for  $WUE_i$ .

526  
527         *Climatic differences between years and between sister taxa when surveyed during*  
528 *flowering*—The qualitative comparison of climatic conditions during the 2007-08 and 2009-10  
529 growing seasons for the 11 sites surveyed in this study indicates that the 2009-10 growing season  
530 was wetter and cooler than 2007-08 (Appendix S8). The mean cumulative precipitation across  
531 sites was 399.4 mm from Nov 2009 - May 2010 vs. 246.5 mm from Nov 2007 - May 2008. The  
532 mean monthly average daily temperature was 10.7 °C for 2009-10 vs. 11.2 for 2007-08. The  
533 wetter and cooler conditions in 2009-10 resulted in lower mean values across sites for minimum

534 VPD (VPD<sub>min</sub>: 2.5 hPa in 2009-10 vs. 3.1 hPa for 2007-08) and maximum VPD (VPD<sub>max</sub>: 14.9  
535 hPa in 2009-10 vs. 17.6 hPa in 2007-08) in the current study relative to Mazer et al. (2010).

536 In the current study, sister taxa experienced different conditions during the month closest  
537 to the time when physiological traits were measured during flowering (Appendix S6). Across the  
538 surveyed populations, *C. exilis* was exposed to 23% lower mean VPD<sub>max</sub> than *C. unguiculata*  
539 (13.3 vs. 17.3 hPa, respectively) and to 55% lower mean VPD<sub>min</sub> (1.11 vs. 2.4 hPa, respectively).  
540 The difference in mean VPD between the two subspecies of *C. xantiana* was both smaller and  
541 less consistent than that between *C. exilis* and *C. unguiculata*. *C. xantiana* ssp. *parviflora*  
542 populations were exposed to a somewhat lower mean VPD<sub>max</sub> than *C. xantiana* ssp. *xantiana*  
543 (22.4 vs. 24.2 hPa, or 7% lower) during flowering, but a higher mean VPD<sub>min</sub> (4.9 vs. 4.3 hPa, or  
544 13% higher).

545

## 546 **DISCUSSION**

547 In each pair of sister taxa examined here, historical herbarium records revealed that each selfing  
548 taxon flowers earlier in the spring than its outcrossing sister taxon, independent of variation  
549 among collection sites in local long-term climatic conditions. Due to these intrinsic phenological  
550 differences, we hypothesized that because local conditions experienced by these *Clarkia* taxa  
551 become increasingly dry as spring progresses, the self-fertilizing taxa experience more mesic  
552 conditions and a lower risk of desiccation during flowering than their outcrossing sister taxa. In  
553 turn, we predicted that this ecological difference would promote relatively fast gas exchange  
554 rates and low WUE<sub>i</sub> in the self-fertilizing taxa due to adaptive evolution, phenotypic plasticity,  
555 or both, and that patterns of phenotypic selection would be consistent with this divergence. Sister  
556 taxa would not necessarily differ physiologically or with respect to phenotypic selection on

557 physiological traits during vegetative growth (i.e., before late-spring drought), unless there is a  
558 strong correlation between physiological rates expressed during vegetative growth and  
559 flowering.

560 Sister taxa differed in all focal physiological traits, but the degree and (in one case) the  
561 direction of divergence depended on life stage (Fig. 6). In general, self-pollinating taxa exhibited  
562 higher gas exchange rates, corroborating our prediction. In addition, the physiological differences  
563 between *C. exilis* and *C. unguiculata* were statistically significant only during flowering,  
564 consistent with our prediction. By contrast, in *C. xantiana*, the two subspecies differed  
565 physiologically more strongly during vegetative growth than during flowering. The expectation  
566 that physiological divergence between sister taxa would be consistent with differences between  
567 them in the strength or direction of phenotypic selection was not upheld in most cases. In 6 of 18  
568 comparisons, patterns of total selection on each trait were concordant with the phenotypic  
569 divergence (or lack thereof) between sister taxa (Table 7). In sum, patterns of selection on  
570 physiological traits did not reliably reflect the observed divergence between sister taxa. This  
571 discrepancy may be particularly common in taxa occupying temporally heterogeneous  
572 environments, underscoring the need for replicated studies of the direction of selection within and  
573 among taxa.

574 The extent to which seasonal drought drives trait evolution and species divergence in  
575 plants has been a topic of longstanding interest. Due to the progressive loss of soil moisture that  
576 characterizes late spring in Mediterranean climates, plant species that are adapted to such  
577 conditions offer an opportunity to identify the traits that evolve in response to the risk of  
578 dehydration. Recent studies of conspecific and interspecific populations exposed to such risk  
579 have tested predictions concerning the phenotypes that will evolve when soil moisture is low,

580 revealing the potential for the rapid evolution of traits that promote drought escape (e.g., relative  
581 fast germination and growth, earlier flowering, and larger leaf size) (Bazzaz, 1979; Heschel and  
582 Riginos, 2005; Franks et al., 2007; Lowry et al., 2008; Wu et al., 2010; Franks, 2011; Kigel et  
583 al., 2011; Heschel et al., 2017; Dickman et al., 2019; Metz et al., 2020) or dehydration avoidance  
584 (e.g., slower growth, higher water use efficiency, decreased transpiration, greater succulence,  
585 higher trichome density, and smaller leaf size) (Arntz and Delph, 2001; Kooyers et al., 2015;  
586 Heschel et al., 2017; Anstett et al., 2021; Burnette and Eckhart, 2021).

587         Studies of the genus *Mimulus* have provided particularly clear cases in which differences  
588 between populations or higher taxa in their exposure to drought are associated with phenotypic  
589 divergence in flowering time, with early flowering associated with more arid conditions (Lowry  
590 et al., 2008, 2009; Kigel et al., 2011; Ivey and Carr, 2012; Wolfe and Tonsor, 2014; Anderson et  
591 al., 2015; Kooyers et al., 2015; Metz et al., 2020). For example, in a greenhouse study of 14 pairs  
592 of coastal and inland populations of *Mimulus guttatus*, Lowry et al. (2008) found that inland  
593 populations, which experience greater summer drought than coastal ones, flowered earlier and  
594 produced smaller leaves and corollas than their coastal counterparts. Evidence that this is the  
595 result of adaptive evolution is provided by a complementary reciprocal transplant experiment, in  
596 which Lowry et al. (2008) found that early flowering contributes to population persistence where  
597 soil moisture is limiting. Consistent with these observations, in a study of selection in two  
598 populations of *M. guttatus* native to a montane habitat with dry midsummer soils vs. a temperate  
599 coastal site, Hall and Willis (2006) found that the difference between sites in the pattern of  
600 selection on flowering time reinforced the phenotypic divergence between the parent  
601 populations.

602           The patterns above are also consistent with subsequent studies. Ivey and Carr (2012)  
603 examined phenotypic selection in greenhouse populations of *M. guttatus* and its self-pollinating,  
604 earlier-flowering, shorter-lived and summer drought-adapted congener, *M. nasutus*. Two  
605 populations per species were raised in wet and in dry conditions, and phenotypic selection was  
606 measured; early flowering contributed most positively to individual fitness where the risk of  
607 dehydration was elevated, particularly in *M. nasutus*. Similarly, in a study of two sympatric  
608 *Mimulus* species that differ in their microhabitat preferences, Ferris and Willis (2018) measured  
609 selection on flowering time, flower size, plant height and leaf shape in a hybrid population  
610 grown in the dry soils of the granite outcrops occupied by the early-flowering *M. laciniatus* and  
611 in the relatively mesic meadows occupied by *M. guttatus*. They found that the direction of  
612 habitat-specific selection was consistent with the differences between species in flowering time  
613 and plant size.

614           Similar patterns have been found at broader geographic scales. In a study of intraspecific  
615 variation in *Arabidopsis thaliana*, 48 lineages were collected in Spain across an elevation  
616 gradient. When raised under controlled conditions, low-elevation genotypes, which were  
617 historically exposed to relatively low precipitation, exhibited earlier bolting, lower net  
618 photosynthesis, slower transpiration rates, and higher WUE than those sourced from high  
619 elevations (Wolfe and Tonsor, 2014). A link between flowering time and adaptation to drought  
620 was also observed among populations of 10 species of annuals distributed across a  
621 Mediterranean-desert rainfall gradient (Kigel et al., 2011); when grown in a common  
622 environment, each species exhibited a positive relationship among populations between  
623 flowering date and rainfall.

624 In *Clarkia*, two studies have measured both divergence between sister taxa in fitness-  
625 related traits and taxon-specific selection on these traits. The first is a comprehensive study of  
626 phenotypic selection on flowering time and leaf and floral traits in *C. xantiana* ssp. *xantiana* and  
627 *C. xantiana* ssp. *parviflora* (Anderson et al., 2015). In this study, 6-12 populations of each  
628 subspecies were raised at three field sites: two sites at which only one of the two subspecies  
629 naturally occurs, and one site located in the contact zone between them. For some traits, variation  
630 among sites in the direction of selection was concordant with predictions based on the  
631 phenotypic divergence between, and the geographic range of, the subspecies. For example,  
632 selection favored individuals with relatively long leaves, higher leaf relative growth rate, and  
633 longer petals in the home site of *C. xantiana* ssp. *xantiana*, which also exhibits higher mean  
634 values of these traits than ssp. *parviflora*. In addition, selection favored earlier flowering only in  
635 the home site of *C. xantiana* ssp. *parviflora*, which flowers earlier on average than ssp. *xantiana*.  
636 In many cases, the strength and significance of the selection coefficients depended on the fitness  
637 component (survival, probability of fruiting, or seed production) examined and, as in the current  
638 study, they were sometimes context or taxon-specific. Another study examined selection and  
639 physiological divergence in nine field populations of *C. unguiculata* and *C. exilis* measured in  
640 situ during vegetative growth and at the onset of flowering (Dudley et al., 2012). While patterns  
641 of selection on photosynthetic rates were generally concordant with inter-specific divergence in  
642 this trait (when populations were pooled), populations and sampling periods differed in the  
643 strength and direction of selection on photosynthetic rate, and there was low concordance  
644 between divergence and selection for either transpiration rate or  $WUE_i$ . The context-specific  
645 relationships observed in these two studies underscore the importance of replicating estimates of  
646 selection gradients and differentials in multiple populations, developmental stages, and years.

647 In all four *Clarkia* taxa examined in the current study, the focal physiological traits had  
648 been recorded previously during vegetative growth and flowering in spring 2008 (Mazer et al.,  
649 2010), when conditions were both drier and warmer than those of the current study (Appendix  
650 S8). In both 2008 and 2010, the magnitude or direction of physiological divergence between  
651 sister taxa changed between vegetative growth and flowering, revealing the lability of these  
652 physiological traits (Table 8). The detection of year-, taxon- and developmental stage-specific  
653 patterns of divergence and selection draws attention to the importance of repeating such studies  
654 when aiming to generate general conclusions concerning their concordance.

655  
656 *Patterns of physiological divergence between sister taxa and their relation to mean flowering time*  
657 — In both pairs of *Clarkia* sister taxa examined here, the herbarium specimen-based analyses  
658 confirmed that when the effects on flowering time of the long-term climatic conditions at the sites  
659 of specimen collection were controlled statistically, the self-pollinating taxa have historically  
660 flowered earlier than their outcrossing sister taxa (Fig. 4). Moreover, the specimen-based analyses  
661 corroborate patterns based on phenological observations in the field of the two subspecies of *C.*  
662 *xantiana* (Moore and Lewis, 1965; Eckhart and Geber, 1999; Eckhart et al., 2004; Guerrant, 2019),  
663 and field and greenhouse observations of *C. exilis* and *C. unguiculata* (Vasek, 1958; Vasek and  
664 Sauer, 1971; Schneider and Mazer, 2016).

665 The particularly early mean collection date for herbarium specimens of *C. exilis* (day 134,  
666 or May 14) relative to *C. unguiculata* (May 24), *C. xantiana* ssp. *parviflora* (May 24) and *C.*  
667 *xantiana* ssp. *xantiana* (May 31; Table 3) and the low values for vapor pressure deficit (VPD)  
668 observed when the two field populations of *C. exilis* in the current study began to flower  
669 (Appendix S6) support the inference that *C. exilis* may be the taxon most likely to escape the

670 desiccating effects of intensifying late-spring drought. If *C. exilis* reliably escapes drought, this  
671 could explain its high photosynthetic and transpiration rates (and low  $WUE_i$ ) relative to *C.*  
672 *unguiculata* (Fig. 6). However, the previous study of physiological divergence in these taxa  
673 (Mazer et al., 2010), reported that *C. exilis* exhibited a faster photosynthetic rate than *C.*  
674 *unguiculata* only during vegetative growth and did not detectably differ from *C. unguiculata* in  
675 transpiration rate during either growth stage (Table 8). This kind of inter-taxon inconsistency was  
676 also observed in *C. xantiana*. In spring 2008, *C. xantiana* ssp. *parviflora* exhibited higher mean  
677 photosynthetic and transpiration rates than ssp. *xantiana* during both vegetative growth and  
678 flowering, but in spring 2010, *C. xantiana* ssp. *parviflora* had a higher transpiration rate than ssp.  
679 *xantiana* during vegetative growth but a lower photosynthetic rate during flowering (Table 8).  
680 Collectively, these patterns indicate that the historical mean flowering dates of these taxa do not  
681 reliably predict that the relatively early-flowering taxa will consistently achieve higher gas  
682 exchange rates than their later-flowering sister taxa. These inconsistencies would not have been  
683 detected in the absence of repeated measures of the physiological performance of these taxa.

684         When pronounced, the patterns of physiological divergence between *C. exilis* and *C.*  
685 *unguiculata* support the prediction that, where drought intensifies as the flowering season  
686 progresses, taxa that flower early (when soil moisture is relatively high and VPD is low) will  
687 exhibit faster gas exchange rates and lower  $WUE_i$  than those that flower late. The patterns of  
688 divergence between the two subspecies of *C. xantiana*, however, are not wholly consistent with  
689 this prediction. Trait divergence recorded in spring 2008 supports the prediction; the earlier-  
690 flowering taxon, *C. xantiana* ssp. *parviflora*, had higher photosynthetic and transpiration rates  
691 than ssp. *xantiana* during flowering (Table 8). However, the patterns of divergence recorded  
692 during flowering in spring 2010 do not; *C. xantiana* ssp. *parviflora* had a lower mean

693 photosynthetic rate than ssp. *xantiana*. The weak support for the “drought escape” hypothesis in  
694 *C. xantiana* may have been due to a lower degree of ecological segregation between the two  
695 subspecies than between *C. unguiculata* and *C. exilis*. For example, among the populations in the  
696 current study, the two subspecies exhibit much more similar values for mean  $VPD_{\min}$  and  $VPD_{\max}$   
697 in the months of their flowering than *C. exilis* and *C. unguiculata* (Appendix S6).

698

699 *Context-specific physiological divergence between sister taxa: variation between developmental*  
700 *stages and years*—In both taxon pairs, the direction or degree of divergence in the focal  
701 physiological traits differed between 2008 and 2010 and/or between developmental stages. In  
702 spring 2008, during vegetative growth, *C. exilis* (self-pollinating) exhibited a significantly higher  
703 photosynthetic rate and higher  $WUE_i$  than *C. unguiculata* (outcrossing), but these differences  
704 disappeared during flowering. In spring 2010, these sister species did not differ in any of the  
705 focal traits during vegetative growth, but during flowering *C. exilis* exhibited higher  
706 photosynthetic and transpiration rates and lower  $WUE_i$  than *C. unguiculata* (Table 8). Despite  
707 *C. exilis*’ early flowering, its  $WUE_i$  relative to *C. unguiculata* changed qualitatively between  
708 years; in 2008, *C. exilis* had higher  $WUE_i$  than *C. unguiculata* during vegetative growth, but in  
709 2010, their relative  $WUE_i$  reversed during flowering.

710 In spring 2008, during vegetative growth, *C. xantiana* ssp. *parviflora* exhibited higher  
711 photosynthetic and transpiration rates, but lower  $WUE_i$ , than ssp. *xantiana*; the differences in gas  
712 exchange rates persisted during flowering, but the difference in  $WUE_i$  disappeared. In spring  
713 2010, during vegetative growth, there was no difference between these subspecies in  
714 photosynthetic rate, but ssp. *parviflora* exhibited a higher mean transpiration rate and lower  $WUE_i$   
715 than ssp. *xantiana*. By contrast, during flowering, ssp. *parviflora* had a lower photosynthetic rate

716 and a higher  $WUE_i$  than *ssp. xantiana*. Of particular note is that, in spring 2010, the two  
717 subspecies of *C. xantiana* differed in  $WUE_i$  during both developmental stages, but the direction of  
718 the divergence changed between vegetative growth and flowering (Fig. 6; Table 7, Table 8).

719 The two taxon pairs differed in the direction of within-season temporal change in  $WUE_i$   
720 during spring 2010. *Clarkia unguiculata* and *C. exilis* exhibited a decline in mean  $WUE_i$  between  
721 vegetative growth and flowering, associated with an increase in their mean transpiration rates.  
722 By contrast, both *C. xantiana ssp. xantiana* and *ssp. parviflora* increased their  $WUE_i$  between  
723 vegetative growth and flowering due to reductions in their mean transpiration rates.

724  
725 *Context-specific concordance between physiological divergence and phenotypic selection*

726 *in each taxon pair*—In this study, selection sometimes reinforced the physiological differences  
727 between sister taxa, with the self-pollinating taxa experiencing phenotypic selection favoring  
728 individuals with a high transpiration rate and low  $WUE_i$  (Concordance Type 2, Table 7).

729 Concordance between sister taxa was also observed in several cases in which neither  
730 physiological divergence nor directional selection were detectable (Concordance Type 1). In a  
731 few cases, the direction of selection differed between sister taxa in a pattern that opposed the  
732 phenotypic difference between them (Discordance Type 1, Table 7). In no case did the type of  
733 concordance or discordance and the direction of statistically significant divergence between  
734 sister taxa remain constant between vegetative growth and flowering. One limitation of the  
735 current study is that our fitness estimates were based entirely on stem biomass, a correlate of  
736 reproductive success. We could not examine the effects of the focal physiological traits on small  
737 seedlings or juveniles, so we cannot assert that these traits did not influence early survivorship  
738 and affect individual fitness. This is a common constraint in studies of phenotypic selection on

739 physiological traits or on traits (e.g., flowering time, flower size, pollen production) that cannot  
740 be measured on juveniles (Lowry et al., 2008; Dudley et al., 2012; Quezada et al., 2017).

741 As a consequence of these inconsistencies and uncertainties, the mechanism(s)  
742 contributing to the observed divergences between sister taxa remains obscure. Phenotypic  
743 plasticity of physiological traits in response to local conditions, responses to direct or total  
744 selection on each physiological trait, and/or indirect responses to selection on unstudied,  
745 correlated traits may all have played a role. Nevertheless, the many cases in which a self-  
746 pollinating taxon exhibited higher photosynthetic or transpiration rates than its outcrossing sister  
747 taxon in 2008 or 2010 (Table 8) support the hypothesis that rapid gas exchange is required to  
748 achieve early maturation, which may be independently favored by selection for any number of  
749 reasons, including the escape from drought-stress. If so, then the divergence in physiological  
750 traits observed in the current and in the previous study (Mazer et al., 2010) may represent the  
751 outcome of adaptive evolution.

752 Phenotypic plasticity in response to conditions during flowering may also have  
753 contributed to the high gas exchange rates and low  $WUE_i$  exhibited by *C. exilis* relative to *C.*  
754 *unguiculata*. In the current study, the conditions at each field site (Appendix S6) indicate that the  
755 populations of *C. exilis* surveyed in flower on April 25-26 were exposed (in April) to a 23%  
756 lower mean  $VPD_{max}$  and a 55% lower mean  $VPD_{min}$  than the populations of *C. unguiculata*  
757 experienced in May (they were surveyed in flower on May 13, 15, 18, and 21). The more mesic  
758 conditions during which *C. exilis* was surveyed may have induced its more rapid gas exchange  
759 rates. The lower photosynthetic rate exhibited by *C. xantiana* ssp. *parviflora* relative to ssp.  
760 *xantiana* cannot be explained as easily by phenotypic plasticity. During flowering in spring  
761 2010, populations of *C. xantiana* ssp. *parviflora* experienced a 13% higher mean  $VPD_{min}$ , but a

762 7% lower mean  $VPD_{max}$  than *ssp. xantiana*. This small environmental difference between them  
763 may account for the lack of divergence in transpiration rate (Table 8), but not *ssp. parviflora*'s  
764 significantly lower photosynthetic rate and higher  $WUE_i$ .

765  
766 *Conclusions*—Snapshots of selection measured in heterogeneous conditions do not  
767 reliably reflect physiological divergence between taxa, highlighting the need for replicated  
768 studies of the factors influencing both divergence and the direction of selection within and  
769 among taxa. Regardless of the mechanism (selection, drift, or plasticity) generating the observed  
770 phenotypic divergence between sister taxa, the current study holds several implications for the  
771 persistence and evolution of these *Clarkia* taxa in response to projected climate warming and  
772 increasingly unpredictable drought (IPCC, 2013). First, earlier flowering of the self-pollinating  
773 taxa may enable them to reproduce more reliably than their outcrossing sister taxa if climate  
774 warming results in higher VPD towards the end of spring. Second, within outcrossing taxa,  
775 selection may progressively favor earlier-flowering genotypes (along with the physiological  
776 traits associated with earlier maturation), which may escape the negative effects of seasonally  
777 increasing VPD. If selection simultaneously operates in sympatric species to increase the  
778 frequency of early-flowering genotypes, then, at the community level, the overlap in flowering  
779 time among taxa may increase, intensifying intra- and interspecific competition for pollinators.  
780 This could provide self-fertilizing taxa with a short-term advantage, but where selfing  
781 populations and lineages fail to produce sufficiently genetically variable offspring due to the  
782 absence of recombinants, their adaptive capacity will be compromised, limiting their ability to  
783 adapt to a rapidly changing environment.

784  
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793

#### 794 **Author Contributions**

795 SJM and LSD were awarded funding from the National Science Foundation to conduct the work  
796 described here; SJM, LSD, and AAH designed and conducted the field work; all authors  
797 contributed to planning the statistical analyses; DJH and SJM conducted the analyses; all authors  
798 discussed and reached consensus on the interpretation of the results; SJM wrote the manuscript,  
799 with editorial contributions from all co-authors.

800

#### 801 **Data Availability**

802 The data sets and the R code used to generate the analyses, figures, and tables included in the  
803 main text and in supplementary material have been deposited in the Dryad Digital Repository:  
804 (<https://doi.org/10.25349/D91318>); the software is published at Zenodo  
805 (<https://doi.org/10.5281/zenodo.5866848>).

806

#### 807 **Supporting Information**

808 Additional supporting information may be found online in the Supporting Information section at  
809 the end of the article.

810  
811 Appendix S1. Annual climatic variables (1951-1980) extracted from ClimateNA for use in  
812 Principal Component Analyses

813 Appendix S2. Climatic parameters contributing to first two principal components for 24 long-  
814 term (1951-1980) mean climatic parameters at the collection sites of the herbarium specimens of  
815 each pair of sister taxa.

816 Appendix S3. Sorted loadings for principal components 1 and 2 for each pair of sister taxa. (a)  
817 *C. unguiculata* and *C. exilis*. (b) *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora*.

818 Appendix S4. Names and GPS coordinates of populations surveyed for physiological traits and  
819 total above-ground stem biomass in 2010.

820 Appendix S5. Dates and sample sizes (n) for physiological data recorded for each taxon, life  
821 history stage, and field population in 2010.

822 Appendix S6. Interpolated monthly climatic parameters extracted from the PRISM database for  
823 each site where populations were surveyed during the 2009-10 growing season.

824 Appendix S7. Application of Bonferroni correction to the tests designed to detect significant  
825 differences between sister taxa (*Clarkia unguiculata* vs. *C. exilis* and *C. xantiana* ssp. *xantiana*  
826 vs. *C. xantiana* ssp. *parviflora*) with respect to physiological traits measured during vegetative  
827 growth and during flowering. All of the 12 tests (3 physiological traits x 2 life history stages x 2  
828 pairs of sister taxa) that were statistically significant at  $\alpha = 0.05$  remained statistically significant  
829 at  $\alpha = 0.05/12 \approx 0.004$ . See Fig. 6 for comparison of means between sister taxa.

830  
831 Appendix S8. Cumulative precipitation and means of monthly climatic parameters from  
832 November 2007-June 2008 and November 2009-June 2010 at each of the 11 field sites where  
833 populations were surveyed in the current study.

834

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1079 Table 1. Predictor variables included in each of four linear models tested using specimen-based  
 1080 data for each taxon pair to detect significant differences between sister taxa in the day of year of  
 1081 specimen collection, controlling for variation in local climatic conditions summarized in the first  
 1082 two principal components. The boldfaced AIC value indicates the model for which the results are  
 1083 presented below (see text). For the analysis of *C. unguiculata* and *C. exilis*, Models 1 and 2 had  
 1084 similar AIC values but the latter model was selected because neither interaction term was  
 1085 statistically significant at  $P < 0.05$ .

	Model 1	Model 2	Model 3	Model 4
Taxon	x	x	x	x
PC1	x	x	x	
PC2	x	x		x
Taxon x PC1	x			
Taxon x PC2	x			
AIC (Taxon pair: <i>unguiculata</i> and <i>exilis</i> )	4692	<b>4691</b>	4714	4790
AIC (Taxon pair: <i>C. xantiana</i> ssp. <i>xantiana</i> and <i>C. xantiana</i> ssp. <i>parviflora</i> )	320.85	319.23	<b>317.24</b>	325.44

1086  
 1087

1088 Table 2. Types of concordance and discordance between physiological divergence between sister  
 1089 taxa and the direction of phenotypic selection on focal trait.

1090

Type of Concordance/Discordance	Phenotypic Divergence Between Sister Taxa?	Difference between sister taxa in selection gradient of the focal trait?
Concordance Type 1	No	No
Concordance Type 2	Yes	Yes; selection gradients <b>reinforce</b> phenotypic divergence
Discordance Type 1	Yes	Yes; selection gradients <b>oppose</b> phenotypic divergence
Discordance Type 2	Yes	No
Discordance Type 3	No	Yes

1091

1092 Table 3. Analysis of variance and summary of linear model designed to test for differences  
 1093 between sister taxa in the day of year of specimen collection (DOY), controlling for variation in  
 1094 PC1 and PC2. Parameter estimates and least squares means of the DOY for each taxon are  
 1095 displayed below the analysis of variance table. a) *Clarkia unguiculata* vs. *C. exilis* (*exilis* is the  
 1096 reference taxon). b) *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora* (*parviflora* is  
 1097 the reference taxon). Significance levels: \*\*\*P < 0.0001; \*\*P < 0.001; \*P < 0.05; ‘.’P < 0.01.  
 1098 CL: Confidence limit. Fig. 3 illustrates the difference between taxon means.  
 1099

1100 a. *Clarkia unguiculata* vs. *C. exilis* — Predictor variables: Taxon + PC1 + PC2  
 1101

Source of variation	df	Sum of Squares	F-value	P-value
Taxon	1	3367	7.71	0.0056**
PC1	1	46526	106.47	< 2.2e-16***
PC2	1	11116	25.44	5.71e-07***
Residuals	767	335175		
Adjusted R <sup>2</sup> = 0.16				
Coefficients				
	Estimate	Std. Error	t-value	Pr(> t )
Intercept	133.82	3.93	34.01	< 2e-16***
Taxon( <i>unguiculata</i> )	11.15	4.02	2.78	0.0056**
PC1	2.34	0.23	10.32	< 2e-16***
PC2	1.45	0.29	5.04	5.71e-07***
	Mean DOY	Std. Error	Lower CL	Upper CL
<i>exilis</i>	134	3.94	126	142
<i>unguiculata</i>	145	0.77	143	146

1102  
 1103 b. *Clarkia xantiana* ssp. *xantiana* vs. ssp. *parviflora* — Predictor variables: Taxon + PC1  
 1104

Source of variation	df	Sum of Squares	F-value	P-value
Taxon	1	630	5.01	0.0289*
PC1	1	1051	8.35	0.0053**
Residuals	62	7807		
Adjusted R <sup>2</sup> = 0.11				
Coefficients				
	Estimate	Std. Error	t-value	Pr(> t )
Intercept	144.63	1.90	76.28	< 2e-16***
Taxon( <i>xantiana</i> )	6.69	2.99	2.24	0.0289*
PC1	1.12	0.39	2.89	0.0053**
	DOY	Std. Error	Lower CL	Upper CL
<i>parviflora</i>	145	1.9	141	148
<i>xantiana</i>	151	2.2	147	156

1105  
 1106

1107 Table 4. Summary of linear models designed to test for differences between sister taxa in  
 1108 climatic conditions represented by the mean values of PC1 or PC2. a) *Clarkia unguiculata* vs. *C.*  
 1109 *exilis* (*exilis* is the reference taxon). b) *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp.  
 1110 *parviflora* (*parviflora* is the reference taxon). Significance levels: \*\*\*\*P < 0.0001; \*\*\*P <  
 1111 0.001; \*\*P < 0.05; \*P < 0.10. CL: Confidence limit.

1112  
 1113 a. *Clarkia unguiculata* vs. *C. exilis* — Response variable: PC2  
 1114

Source of variation	df	Sum of Squares	F-value	P-value
Taxon	1	329.6	48.66	6.57e-12****
Residuals	769	5209.6		
Adjusted R <sup>2</sup> = 0.06				
Coefficients	Estimate	Std. Error	t-value	P-value
Intercept	-3.25	0.475	-6.84	1.63e-11****
Taxon( <i>unguiculata</i> )	3.38	0.485	6.98	6.57e-12****
	Mean value of PC2	Std. Error	Lower CL	Upper CL
<i>exilis</i>	-3.25	0.475	-4.18	-2.32
<i>unguiculata</i>	0.13	0.10	-0.06	0.32

1115  
 1116 b. *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora* — Response variable: PC1  
 1117

Source of variation	df	Sum of Squares	F-value	P-value
Taxon	1	103.9	8.33	0.0053**
Residuals	63	785.4		
Adjusted R <sup>2</sup> = 0.10				
Coefficients	Estimate	Std. Error	t-value	P-value
Intercept	1.10	0.581	1.89	0.06
Taxon( <i>xantiana</i> )	-2.55	0.884	-2.89	0.0053**
	Mean value of PC1	Std. Error	Lower CL	Upper CL
<i>parviflora</i>	1.10	0.580	-0.06	2.26
<i>xantiana</i>	-1.45	0.667	-2.79	-0.12

1118

1119 Table 5. Least squares means (standard error, n) of photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  
 1120 transpiration ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , and WUE ( $\mu\text{mol}_{\text{CO}_2} \text{ mol}_{\text{H}_2\text{O}}^{-1} \times 0.0001$ ) for each taxon, controlling  
 1121 for node number and Tleaf, and with population treated as a random effect. a) Trait means  
 1122 during vegetative growth. b) Trait means during flowering. Boldfaced means with distinct  
 1123 superscripts differ between sister taxa during a given developmental stage.

1124  
 1125  
 1126

a. Vegetative Growth

Response variable	<i>unguiculata</i>	<i>exilis</i>	<i>xantiana</i>	<i>parviflora</i>
Photo	20.7 (2.3, 306)	21.3 (3.4, 116)	30 (2, 249)	31.2 (2.3, 226)
Trans	0.00965 (0.00073, 306)	0.00902 (0.0011, 116)	<b>0.0154<sup>b</sup></b> (0.001, 249)	<b>0.0202<sup>a</sup></b> (0.0012, 226)
WUE <sub>i</sub>	0.238 (0.02, 306)	0.255 (0.03, 116)	<b>0.212<sup>a</sup></b> (0.015, 249)	<b>0.166<sup>b</sup></b> (0.017, 226)

1127

1128 b. Flowering

Response variable	<i>unguiculata</i>	<i>exilis</i>	<i>xantiana</i>	<i>parviflora</i>
Photo	<b>19.1<sup>b</sup></b> (2.7, 286)	<b>25.2<sup>a</sup></b> (3.8, 151)	<b>20.8<sup>a</sup></b> (2.4, 280)	<b>18.7<sup>b</sup></b> (2.8, 196)
Trans	<b>0.0102<sup>b</sup></b> (0.0015, 286)	<b>0.0152<sup>a</sup></b> (0.0022, 151)	0.00865 (0.00098, 280)	0.00801 (0.0012, 196)
WUE <sub>i</sub>	<b>0.222<sup>a</sup></b> (0.0051, 286)	<b>0.165<sup>b</sup></b> (0.0049, 151)	<b>0.294<sup>b</sup></b> (0.047, 280)	<b>0.338<sup>a</sup></b> (0.056, 196)

1129

Table 6. Selection coefficients and 95% CIs for predictor variables in each of 5 models per taxon pair and growth stage (vegetative vs. flowering). For each model, filled cells in its column indicate the predictor variables included in the model. a) Vegetative growth, *Clarkia unguiculata* vs. *C. exilis*. b) Vegetative growth, *Clarkia xantiana* ssp. *xantiana* vs. ssp. *parviflora*. d) Flowering, *Clarkia unguiculata* vs. *C. exilis*. d) Flowering, *Clarkia xantiana* ssp. *xantiana* vs. ssp. *parviflora*. Units for physiological traits are as follows: Photosynthesis,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; Transpiration,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ; instantaneous water use efficiency ( $\text{WUE}_i$ ,  $\mu\text{mol CO}_2/\text{mol H}_2\text{O} \times 0.0001$ ). Significance: \*  $p < 0.10$ . \*\*  $p < 0.05$ . Models A, B, and C estimate total phenotypic selection on each physiological trait; Models D and E measure direct selection on photosynthetic and transpiration rates, with the effect of each variable estimated independent of the effect of the other.

a. *Clarkia unguiculata* vs. *C. exilis* (*unguiculata* is the baseline), Vegetative growth stage

Predictor	Model A	Model B	Model C	Model D	Model E
Photo	0.158** (0.045, 0.265)			0.201** (0.088, 0.324)	0.220** (0.095, 0.346)
Trans		-0.020 (-0.139, 0.057)		-0.106** (-0.219, -0.032)	-0.102** (-0.220, -0.020)
WUE <sub>i</sub>			0.175** (0.065, 0.298)		
Taxon <i>exilis</i>	0.220** (0.032, 0.524)	0.146* (-0.007, 0.421)	0.205** (0.039, 0.500)	0.284** (0.090, 0.647)	0.287** (0.082, 0.648)
Taxon x Photo	-0.131 (-0.381, 0.147)			-0.347 (-0.779, 0.131)	-0.342 (-0.765, 0.105)
Taxon x Trans		0.293* (-0.037, 0.645)		0.453* (-0.043, 0.951)	0.418* (-0.030, 0.901)
Taxon x WUE <sub>i</sub>			-0.344* (-0.663, 0.008)		
Photo x Trans					-0.075 (-0.156, 0.060)
Node number	0.081** (0.054, 0.113)	0.090** (0.068, 0.127)	0.086** (0.062, 0.116)	0.077** (0.053, 0.109)	0.076** (0.052, 0.108)
Tleaf	0.031** (0.007, 0.093)	0.032** (0.008, 0.096)	0.039** (0.025, 0.101)	0.035** (0.020, 0.109)	0.036** (0.017, 0.103)

Table 6 (continued)

a. *Clarkia xantiana* ssp. *xantiana* vs. ssp. *parviflora* (*xantiana* is the baseline), Vegetative growth stage

Predictor	Model A	Model B	Model C	Model D	Model E
Photo	0.153** (0.027, 0.279)			0.201** (0.044, 0.359)	0.207** (0.046, 0.366)
Trans		0.036 (-0.072, 0.140)		-0.089 (-0.221, 0.041)	-0.096 (-0.228, 0.035)
WUE <sub>i</sub>			0.150** (0.029, 0.271)		
Taxon <i>parviflora</i>	-0.600** (-0.890, -0.324)	-0.619** (-0.913, -0.340)	-0.652** (-0.956, -0.358)	-0.615** (-0.907, -0.337)	-0.617** (-0.907, -0.338)
Taxon x Photo	-0.038 (-0.200, 0.125)			-0.072 (-0.261, 0.118)	-0.074 (-0.261, 0.117)
Taxon x Trans		0.025 (-0.117, 0.171)		0.068 (-0.094, 0.229)	0.074 (-0.091, 0.238)
Taxon x WUE <sub>i</sub>			-0.095 (-0.234, 0.044)		
Photo x Trans					0.023 (-0.043, 0.089)
Node number	0.137** (0.094, 0.183)	0.142** (0.098, 0.189)	0.137** (0.092, 0.187)	0.134** (0.090, 0.182)	0.133** (0.090, 0.180)
Tleaf	0.004 (-0.022, 0.033)	-0.003 (-0.029, 0.025)	0.013 (-0.016, 0.043)	0.009 (-0.019, 0.039)	0.010 (-0.018, 0.040)

Table 6 (continued)

b. *Clarkia unguiculata* vs. *C. exilis* (*unguiculata* is the baseline), Flowering

Predictor	Model A	Model B	Model C	Model D	Model E
Photo	0.131** (0.036, 0.228)			0.089 (-0.027, 0.207)	0.089 (-0.027, 0.204)
Trans		0.142** (0.012, 0.282)		0.083 (-0.078, 0.260)	0.078 (-0.070, 0.249)
WUE <sub>i</sub>			0.005 (-0.078, 0.083)		
Taxon <i>exilis</i>	-0.985** (-1.483, -0.474)	-0.927** (-1.370, -0.434)	-1.068** (-1.588, -0.547)	-0.873** (-1.281, -0.333)	-0.885** (-1.311, -0.350)
Taxon x Photo	0.038 (-0.112, 0.183)			0.153 (-0.034, 0.339)	0.153 (-0.038, 0.343)
Taxon x Trans		-0.204** (-0.384, -0.041)		-0.238** (-0.462, -0.033)	-0.228** (-0.462, -0.026)
Taxon x WUE <sub>i</sub>			0.212** (0.025, 0.403)		
Photo x Trans					0.019 (-0.090, 0.093)
Node number	0.077** (0.057, 0.100)	0.082** (0.062, 0.104)	0.080** (0.059, 0.103)	0.078** (0.058, 0.100)	0.077** (0.058, 0.100)
Tleaf	0.019 (-0.034, 0.069)	0.008 (-0.040, 0.054)	0.018 (-0.033, 0.067)	0.031 (-0.013, 0.082)	0.028 (-0.017, 0.082)

c. *Clarkia xantiana* ssp. *xantiana* vs. ssp. *parviflora* (*xantiana* is the baseline), Flowering

Predictor	Model A	Model B	Model C	Model D	Model E
Photo	0.037 (-0.067, 0.141)			-0.009 (-0.120, 0.105)	-0.008 (-0.121, 0.106)
Trans		0.079 (-0.039, 0.194)		0.083 (-0.047, 0.212)	0.082 (-0.039, 0.203)
WUE <sub>i</sub>			-0.053 (-0.172, 0.073)		
Taxon <i>parviflora</i>	-0.675** (-0.841, -0.496)	-0.659** (-0.815, -0.493)	-0.677** (-0.838, -0.502)	-0.657** (-0.823, -0.483)	-0.655** (-0.826, -0.474)
Taxon x Photo	0.017 (-0.171, 0.207)			0.063 (-0.179, 0.305)	0.063 (-0.179, 0.307)
Taxon x Trans		-0.044 (-0.182, 0.090)		-0.083 (-0.262, 0.091)	-0.085 (-0.283, 0.103)
Taxon x WUE <sub>i</sub>			-0.069 (-0.286, 0.153)		
Photo x Trans					0.005 (-0.085, 0.097)
Node number	0.077** (0.055, 0.099)	0.078** (0.056, 0.100)	0.080** (0.058, 0.103)	0.078** (0.055, 0.100)	0.078** (0.054, 0.100)
Tleaf	-0.001 (-0.024, 0.020)	-0.002 (-0.025, 0.018)	-0.006 (-0.028, 0.015)	-0.002 (-0.026, 0.021)	-0.002 (-0.026, 0.020)

Table 7. Occurrences and types of concordance and discordance between the direction of phenotypic divergence between sister taxa in gas exchange rates and  $WUE_i$ , and the taxon-specific selection gradients; see Table 2 for the definitions of each type of concordance and discordance. a) *Clarkia unguiculata* (*Cu*) vs. *C. exilis* (*Ce*). b) *Clarkia xantiana* ssp. *xantiana* (*Cxx*) vs. ssp. *parviflora* (*Cxp*). Cells indicate whether sister taxa differ in mean phenotype (> and < indicate the taxon with the higher phenotypic value; see Fig. 4) and the type of concordance or discordance observed between the directions of phenotypic divergence and selection in each taxon pair.

a. *Clarkia unguiculata* vs. *C. exilis*

	<b>Total selection:</b>	Vegetative stage: divergence vs. selection	Flowering stage: divergence vs. selection	Flowering stage divergence vs. Vegetative stage selection
Photosynthesis	<b>Concordance Type 1</b>	Discordance Type 3 $Ce = Cu$	Discordance Type 2 $Ce > Cu$	Discordance Type 2 $Ce > Cu$
Transpiration	Discordance Type 3 $Ce = Cu$	Discordance Type 1 $Ce > Cu$	Discordance Type 1 $Ce > Cu$	<b>Concordance Type 2</b> $Ce > Cu$
$WUE_i$	Discordance Type 3 $Ce = Cu$	Discordance Type 1 $Ce < Ce$	Discordance Type 1 $Ce < Ce$	<b>Concordance Type 2</b> $Ce < Cu$

**Direct selection:**

Photosynthesis	<b>Concordance Type 1</b>	Discordance Type 3 $Ce = Cu$	Discordance Type 2 $Ce > Cu$	Discordance Type 2 $Ce > Cu$
Transpiration	Discordance Type 3 $Ce = Cu$	Discordance Type 1 $Ce > Cu$	Discordance Type 1 $Ce > Cu$	<b>Concordance Type 2</b> $Ce > Cu$

Table 7 (continued)

b. *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora*

<b>Total selection:</b>	Vegetative stage: divergence vs. selection	Flowering stage: divergence vs. selection	Flowering stage divergence vs. Vegetative stage selection
Photosynthesis	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$	Discordance Type 2 $C_{xp} < C_{xx}$	Discordance Type 2 $C_{xp} < C_{xx}$
Transpiration	Discordance Type 2 $C_{xp} > C_{xx}$	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$
WUE <sub>i</sub>	Discordance Type 2 $C_{xp} < C_{xx}$	Discordance Type 2 $C_{xp} > C_{xx}$	Discordance Type 2 $C_{xp} > C_{xx}$
<b>Direct selection:</b>			
Photosynthesis	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$	Discordance Type 2 $C_{xp} < C_{xx}$	Discordance Type 2 $C_{xp} < C_{xx}$
Transpiration	Discordance Type 2 $C_{xp} > C_{xx}$	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$	<b>Concordance Type 1</b> $C_{xp} = C_{xx}$

Table 8. Divergence in gas exchange rates and  $WUE_i$  between sister taxa recorded in the current study and in Mazer et al. (2010). Within each cell, the operator (>, <) indicates the direction of significant differences reported between sister taxon means. Values in green illustrate the case in which the direction of divergence between sister taxa differs qualitatively between studies (2008 vs. 2010). Values shaded in yellow illustrate the case in which the direction of divergence between sister taxa differs qualitatively between life stages. Values shaded in blue illustrate the case in which the direction of divergence between sister taxa differs qualitatively between life stages and studies. In all other cases, divergence between sister taxa either does not change between life stages or studies, or changes from statistically significant to non-significant ( $\alpha = 0.05$ ).

<i>Clarkia unguiculata</i> vs. <i>C. exilis</i>				
	Mazer et al. (2010)		Current study	
	Field season: 2007-08		Field season: 2009-10	
	Vegetative	Flowering	Vegetative	Flowering
Photosynthesis	<b>Ce &gt; Cu</b>	Ce = Cu	Ce = Cu	<b>Ce &gt; Cu</b>
Transpiration	Ce = Cu	Ce = Cu	Ce = Cu	<b>Ce &gt; Cu</b>
$WUE_i$	<b>Ce &gt; Cu</b>	Ce = Cu	Ce = Cu	<b>Ce &lt; Cu</b>

<i>Clarkia xantiana</i> ssp. <i>xantiana</i> vs. <i>C. xantiana</i> ssp. <i>parviflora</i>				
	Mazer et al. (2010)		Current study	
	Field season: 2007-08		Field season: 2009-10	
	Vegetative	Flowering	Vegetative	Flowering
Photosynthesis	<b>Cxp &gt; Cxx</b>	<b>Cxp &gt; Cxx</b>	Cxp = Cxx	<b>Cxp &lt; Cxx</b>
Transpiration	<b>Cxp &gt; Cxx</b>	<b>Cxp &gt; Cxx</b>	<b>Cxp &gt; Cxx</b>	Cxp = Cxx
$WUE_i$	<b>Cxp &lt; Cxx</b>	Cxp = Cxx	<b>Cxp &lt; Cxx</b>	<b>Cxp &gt; Cxx</b>

## FIGURE LEGENDS

Fig. 1. Photographs of the study species. A. *Clarkia unguiculata*. B. *C. exilis*. C. *C. xantiana* ssp. *xantiana*. D. *C. xantiana* ssp. *parviflora*. Within each taxon, mean flower size differs within and among field populations, but autogamous taxa consistently produce smaller petals than their pollinator-dependent sister taxa. The area of individual petals of *C. unguiculata* (mean = 0.52 cm<sup>2</sup>, SD = 0.20, Range = 0.15-1.14, n = 1302 petals [two per plant] sampled from 12 populations in 2001, unpublished data) is larger than that of *C. exilis* (mean = 0.41 cm<sup>2</sup>, SD = 0.09, Range = 0.17-0.68, n = 1000 petals [2 per plant] sampled from 11 populations in 2001, unpublished data). The area of individual petals produced by *C. xantiana* ssp. *xantiana* (mean = 0.93 cm<sup>2</sup>, SD = 0.24, Range = 0.47-1.74, n = 366 individuals [one to four petals per plant] sampled from 7 populations in 2001; unpublished data) is larger than that of *C. xantiana* ssp. *parviflora* (mean = 0.46 cm<sup>2</sup>, SD = 0.16, Range = 0.15-0.99, n = 180 individuals [one to four petals per plant] sampled from 4 populations in 2001, unpublished data). Photo credit: SJM.

Fig. 2. Distributions of georeferenced collection sites of herbarium specimens (with and without topographic features) analyzed to detect climatic differences between sister taxa with respect to the mean long-term conditions that they occupy. A. *Clarkia unguiculata* (blue points) and *C. exilis* (red points); B. *C. xantiana* ssp. *xantiana* (blue points) and *C. xantiana* ssp. *parviflora* (red points). The bounded box in the right-hand maps indicates the region from which field populations were sampled for this study (see Fig. 3).

Fig. 3. Map of populations surveyed for physiological traits and individual fitness in 2010.

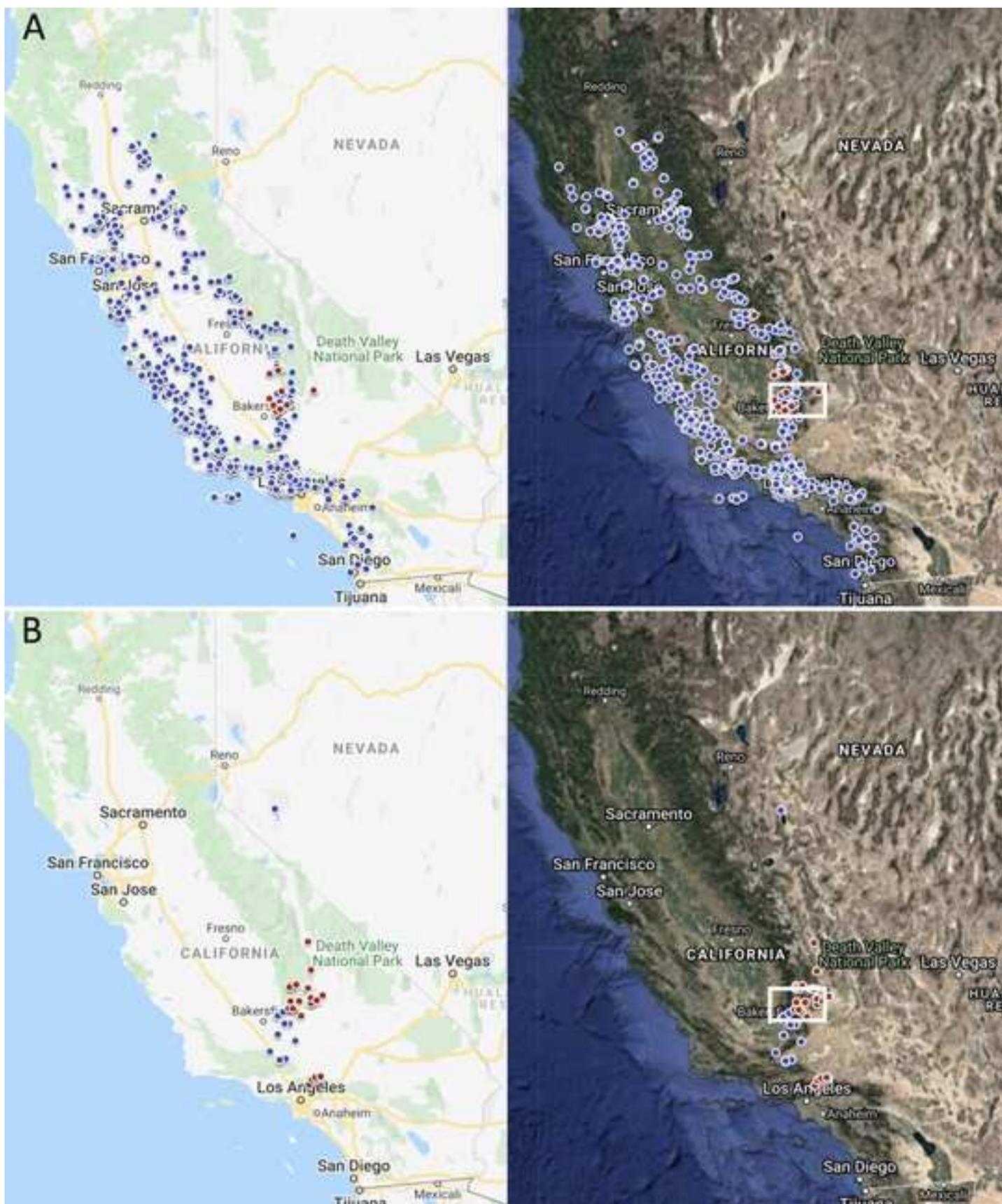
Fig. 4. Box plots illustrating divergence between sister taxa in the day of year of specimen collection (DOY), independent of variation in long-term climatic conditions among collection sites, based on herbarium data. A. *Clarkia unguiculata* vs. *C. exilis* B. *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora*.

Fig. 5. Occupation of climate space by sister taxa, as represented by the first two principal components. A. *Clarkia unguiculata* and *C. exilis*. B. *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora*. Due to the much broader geographic distribution of *Clarkia unguiculata* relative to *C. exilis* (Fig. 2), the former occupies a much wider range of climatic conditions represented by PC2.

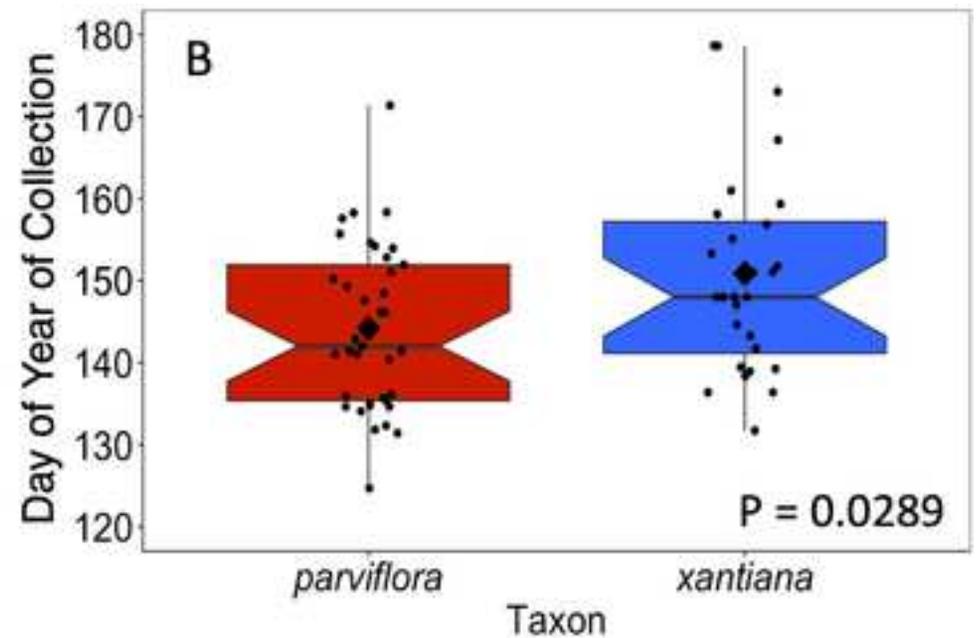
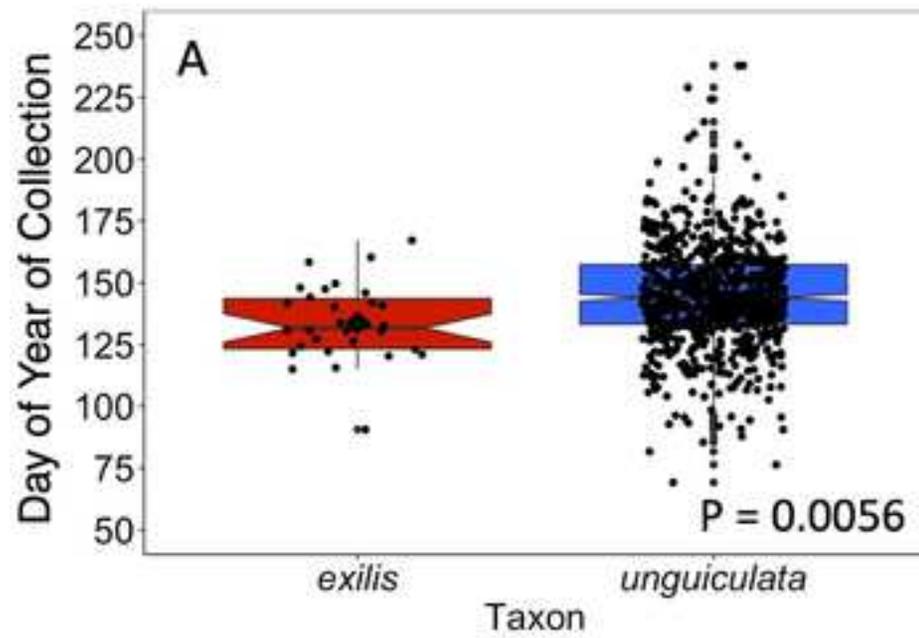
Fig. 6. Differences in physiological traits between sister taxa; each pair of taxa was analyzed separately. A. *Clarkia unguiculata* vs. *C. exilis*. B. *Clarkia xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora*. The significance of the differences between taxa was assessed using 90% and 95% bootstrap confidence intervals for the taxon effects. Single asterisks (\*) indicate cases where the 90% CI for the taxon effect does not contain zero, while the double asterisks (\*\*) indicate cases where the 95% CI for the taxon effect does not contain zero. All tests for which the difference between the means of the two sister taxa was significant at  $\alpha = 0.05$  withstood Bonferroni correction (Appendix S7). The small vertical bars in each violin plot indicate the medians. Units for physiological traits are as follows: Photosynthesis,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; Transpiration,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ; instantaneous water use efficiency ( $\text{WUE}_i$ ,  $\mu\text{mol CO}_2/\text{mol H}_2\text{O} \times 0.0001$ ).

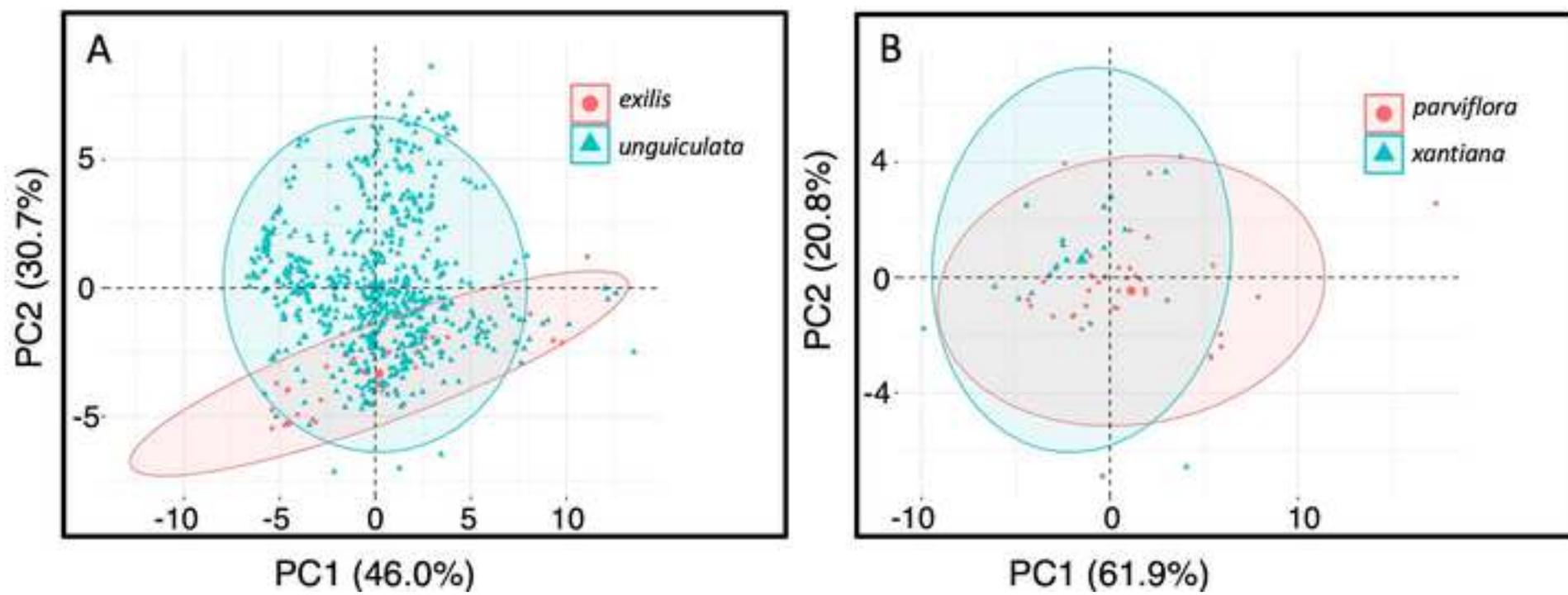
Fig. 7. Taxon-specific selection gradients during vegetative growth and flowering for photosynthesis, transpiration, and  $WUE_i$ . a. Total selection gradients for each trait (Models A, B, C). b. Direct selection gradients for photosynthesis and transpiration (Model D). The significance of the differences between taxa in the slope of the relationship between the physiological trait and biomass (i.e., the Taxon x Trait interaction) was assessed using 90% (\*) and 95% (\*\*) bootstrap confidence intervals for the taxon effects. The shaded regions indicate the range of slopes captured by the 95% bootstrap confidence interval for the gradient. Units for physiological traits are as follows: Photosynthesis,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; Transpiration,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ; instantaneous water use efficiency ( $WUE_i$ ,  $\mu\text{mol CO}_2/\text{mol H}_2\text{O} \times 0.0001$ ).

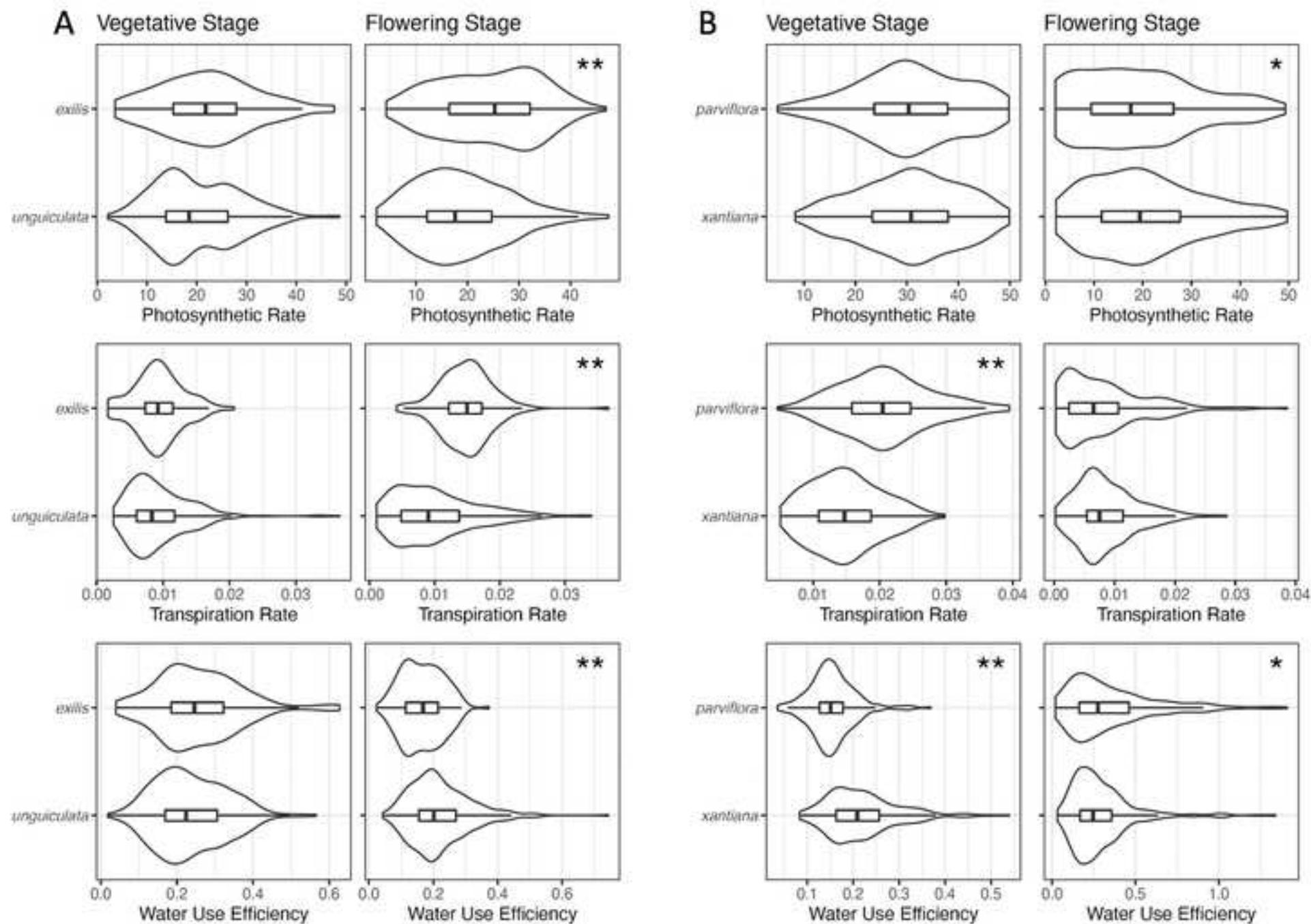


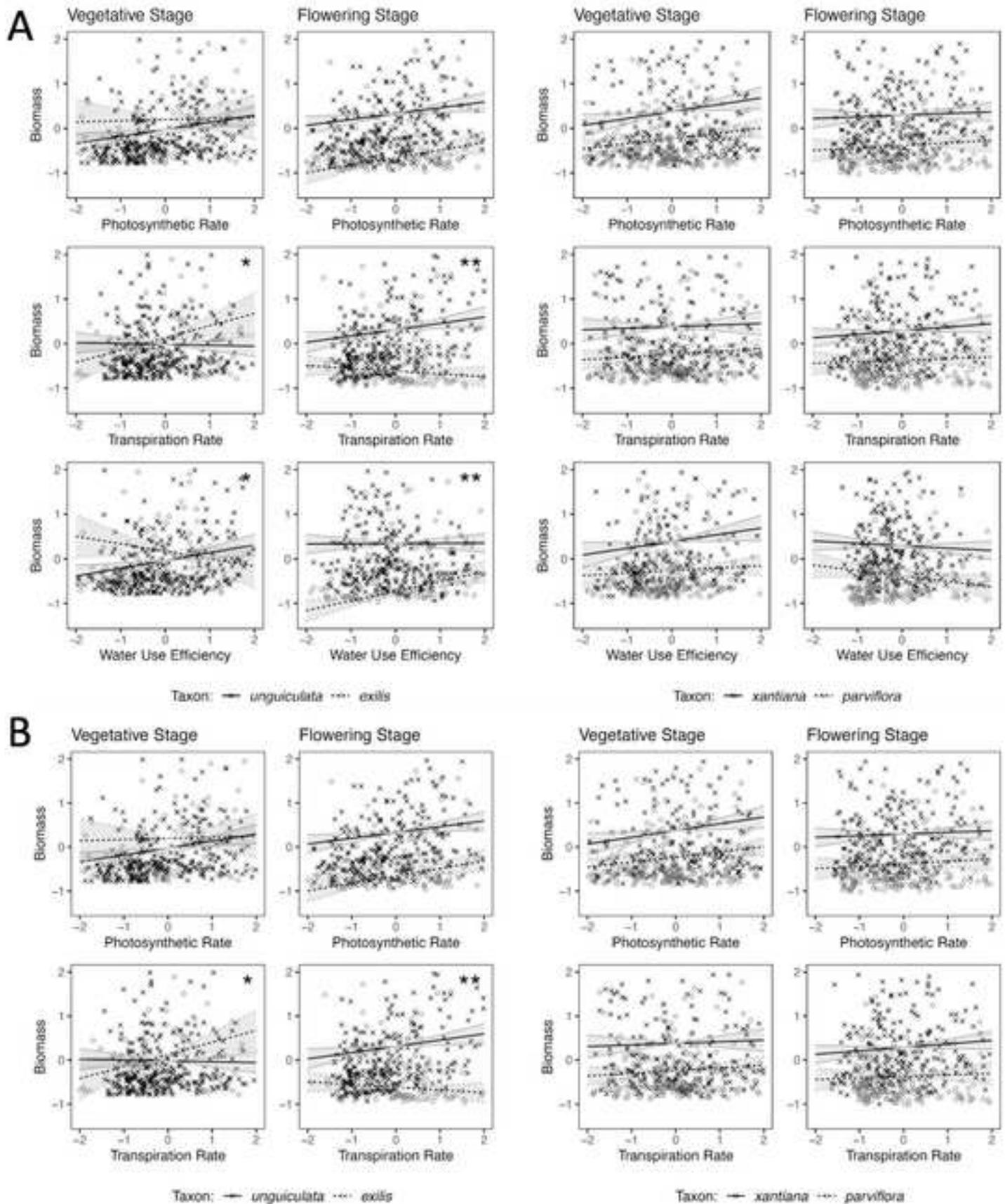












## Mazer et al.—American Journal of Botany 2022—Appendix S1

Appendix S1. Summary of annual climatic variables extracted from ClimateNA for use in Principal Component Analyses conducted to detect differences between sister taxa in the chronic climatic conditions (1951-1980) occupied by specimens collected and archived in the Consortium of California Herbaria. List obtained from the Help file of ClimateNA ([climatena.ca/Help](http://climatena.ca/Help)). The principal components derived from these variables were also used in models designed to detect significant differences between sister taxa in the day of year of specimen collection, independent of local climatic conditions.

***Directly calculated annual variables:***

MAT	mean annual temperature (°C),
MWMT	mean warmest month temperature (°C),
MCMT	mean coldest month temperature (°C),
TD	temperature difference between MWMT and MCMT, or continentality (°C),
MAP	mean annual precipitation (mm),
MSP	May to September precipitation (mm),
AHM	annual heat-moisture index $(MAT+10)/(MAP/1000)$
SHM	summer heat-moisture index $((MWMT)/(MSP/1000))$

***Derived annual variables:***

DD<0	degree-days below 0°C, chilling degree-days
DD>5	degree-days above 5°C, growing degree-days
DD<18	degree-days below 18°C, heating degree-days
DD>18	degree-days above 18°C, cooling degree-days
NFFD	the number of frost-free days
FFP	frost-free period
bFFP	the day of the year on which FFP begins
eFFP	the day of the year on which FFP ends
PAS	precipitation as snow (mm). For individual years, it covers the period between August in the previous year and July in the current year.
EMT	extreme minimum temperature over 30 years
EXT	extreme maximum temperature over 30 years
Eref	Hargreaves reference evaporation (mm)
CMD	Hargreaves climatic moisture deficit (mm)
RH	mean annual relative humidity (%)
CMI	Hogg's climate moisture index (mm)
DD1040	Degree-days above 10°C and below 40°C

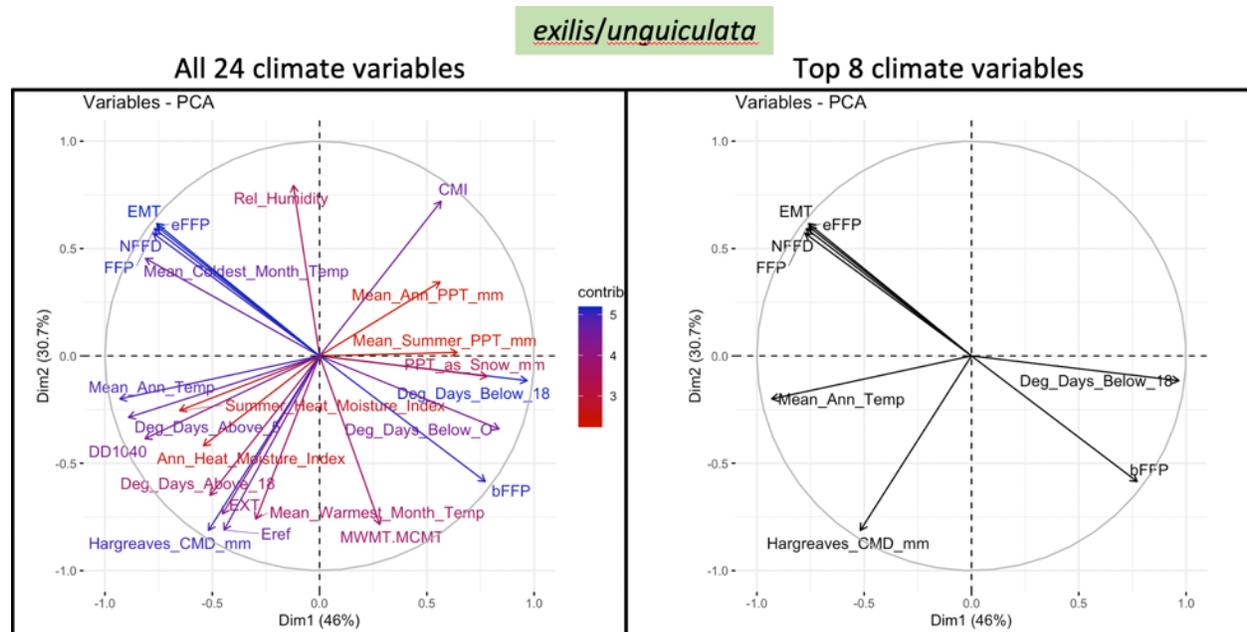
**Note: the following variable was omitted from the PC analysis due to too many missing values**

MAR: Mean annual solar radiation

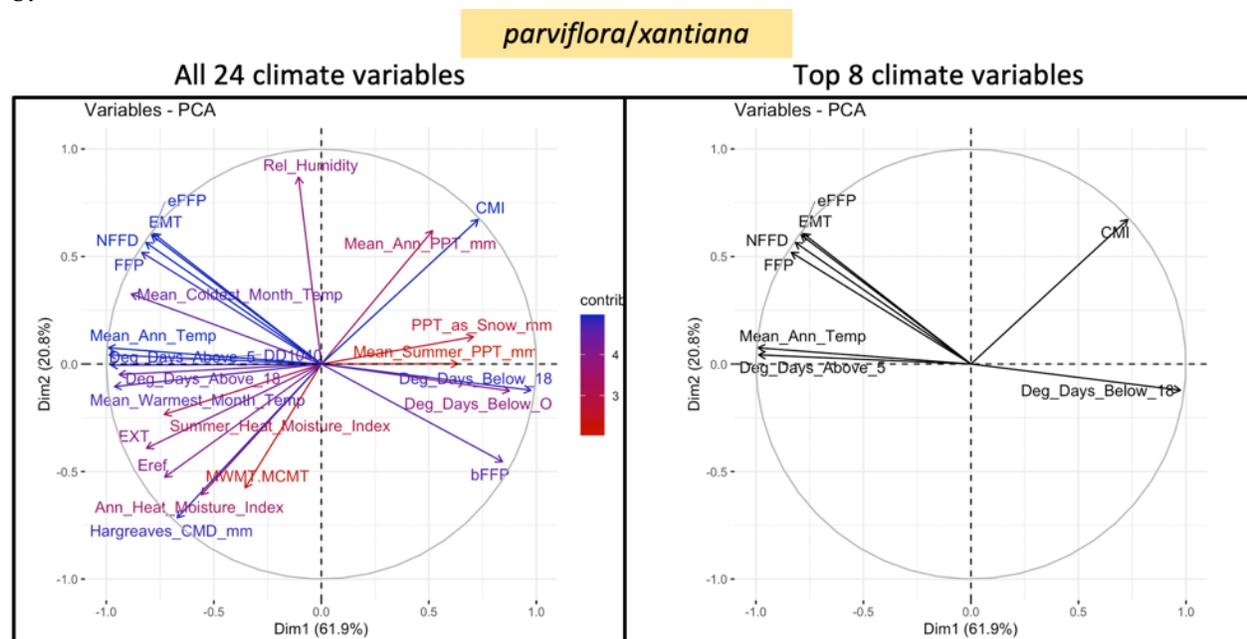
Mazer et al.—American Journal of Botany 2022—Appendix S2

Appendix S2. Principal components based on 24 long-term (1951-1980) mean climatic parameters at the collection sites of the herbarium specimens of each pair of sister taxa. PCA graphs on the left illustrate the relative contributions of all climatic variables; PCA graphs on the right illustrate the contributions of the 8 most important variables. (a) *C. unguiculata* and *C. exilis*. (b) *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora*.

a.



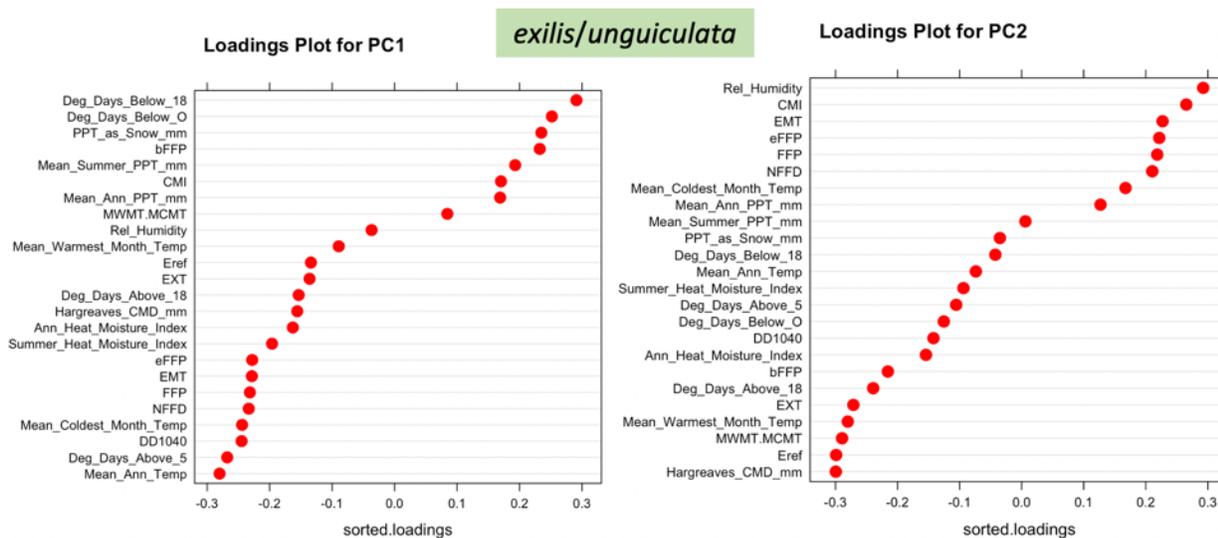
b.



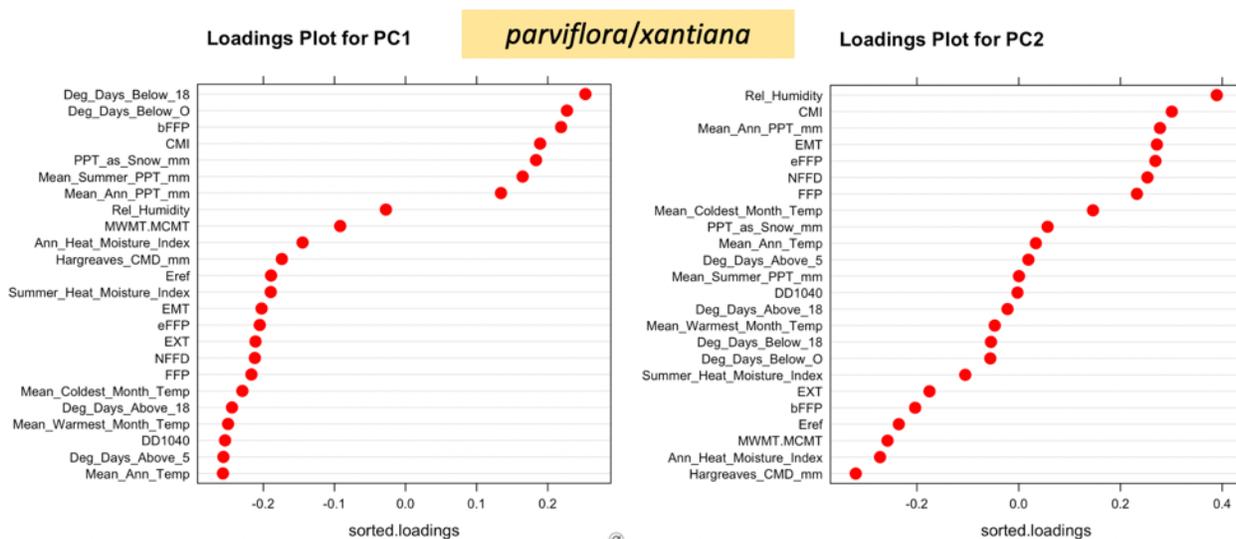
Mazer et al.—American Journal of Botany 2022—Appendix S3

Appendix S3. Sorted loadings for principal components 1 and 2 for each pair of sister taxa. (a) *C. unguiculata* and *C. exilis*. (b) *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora*.

a.



b.



## Mazer et al.—American Journal of Botany 2022—Appendix S4

Appendix S4. Names and GPS coordinates of populations surveyed for physiological traits and total above-ground stem biomass in 2010.

Locality Name	Taxon	Elevation (m)	Latitude (N)	Longitude (W)
Willow Springs	<i>exilis</i>	365	35.670	-118.902
Stark Creek	<i>exilis</i>	457	35.474	-118.726
Stark Creek	<i>unguiculata</i>	457	35.474	-118.726
Live Oak	<i>unguiculata</i>	430	35.479	-118.749
Democrat Springs Campground	<i>unguiculata</i>	590	35.531	-118.663
Democrat Springs Campground	<i>xantiana</i>	590	35.531	-118.663
China Gardens	<i>unguiculata</i>	641	35.533	-118.646
Borel Road	<i>xantiana</i>	707	35.584	-118.522
Camp 3	<i>xantiana</i>	896	35.812	-118.453
Fay Ranch Road	<i>parviflora</i>	927	35.700	-118.304
Sawmill Road	<i>parviflora</i>	1003	35.678	-118.481
Sawmill Road	<i>xantiana</i>	1217	35.675	-118.510
Long Valley	<i>parviflora</i>	1628	35.815	-118.091

## Mazer et al.—American Journal of Botany 2022—Appendix S5

Appendix S5. Dates and sample sizes (n) for physiological data recorded for each taxon, life history stage, and site in 2010. The number of days between the vegetative and flowering stage surveys is also displayed.

Dates of gas exchange in 2010	Stage	Population	n	Date of Gas Exchange Measurement (Yr-month-day)	# of Days between Veg & Rep Surveys
<i>exilis</i>	Vegetative	Stark Creek	57	2010-04-04	21
<i>exilis</i>	Reproductive	Stark Creek	72	2010-04-25	
<i>exilis</i>	Vegetative	Willow Springs	67	2010-04-09	17
<i>exilis</i>	Reproductive	Willow Springs	85	2010-04-26	
<i>unguiculata</i>	Vegetative	China Gardens	89	2010-05-05	13
<i>unguiculata</i>	Reproductive	China Gardens	68	2010-05-18	
<i>unguiculata</i>	Vegetative	Democrat Springs	90	2010-04-10	41
<i>unguiculata</i>	Reproductive	Democrat Springs	86	2010-05-21	
<i>unguiculata</i>	Vegetative	Live Oak	86	2010-03-26	48
<i>unguiculata</i>	Reproductive	Live Oak	89	2010-05-13	
<i>unguiculata</i>	Vegetative	Stark Creek	98	2010-04-24	21
<i>unguiculata</i>	Reproductive	Stark Creek	81	2010-05-15	
<i>parviflora</i>	Vegetative	Fay Ranch Road	75	2010-05-03	17
<i>parviflora</i>	Reproductive	Fay Ranch Road	58	2010-05-20	
<i>parviflora</i>	Vegetative	Long Valley	80	2010-05-09	34
<i>parviflora</i>	Reproductive	Long Valley	73	2010-06-12	
<i>parviflora</i>	Vegetative	Sawmill Road	79	2010-05-04	27
<i>parviflora</i>	Reproductive	Sawmill Road	68	2010-05-31	
<i>xantiana</i>	Vegetative	Borel Road	54	2010-04-23	42
<i>xantiana</i>	Reproductive	Borel Road	82	2010-06-04	
<i>xantiana</i>	Vegetative	Camp 3	73	2010-04-18	44
<i>xantiana</i>	Reproductive	Camp 3	75	2010-06-01	
<i>xantiana</i>	Vegetative	Democrat Springs	87	2010-05-07	37
<i>xantiana</i>	Reproductive	Democrat Springs	77	2010-06-13	
<i>xantiana</i>	Vegetative	Sawmill Road	86	2010-05-01	45
<i>xantiana</i>	Reproductive	Sawmill Road	72	2010-06-15	

## Mazer et al.—American Journal of Botany 2022—Appendix S6

Appendix S6. Interpolated monthly climatic parameters extracted from the PRISM database for each population surveyed during the 2009-10 growing season. Months in red font indicate the month corresponding most closely to the month during which physiological traits were recorded during flowering.

<i>C. exilis</i> Willow Springs		Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
Date (year-month)	ppt (mm)						
2009-11	13.22	3.6	11.4	19.3	1.7	1.32	14.87
2009-12	69.49	2	7.8	13.7	1.7	0.61	8.04
2010-01	64.69	3.3	8.7	14.1	3.3	0.43	7.57
2010-02	77.18	4.5	10.1	15.6	5.5	0.39	7.62
2010-03	15.04	4.6	11.6	18.7	4.1	0.91	13.39
2010-04	58.97	5.2	12.5	19.7	4.8	0.85	14.37
2010-05	8.15	7.2	15.5	23.8	3.9	1.77	22.12
<i>C. exilis</i> Stark Creek		Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
Date (year-month)	ppt (mm)						
2009-11	14.09	5.6	12	18.4	-2.8	3.6	16.16
2009-12	64.39	1.5	6.9	12.3	-3.1	1.73	8.82
2010-01	64	3.2	8.7	14.1	-1.3	1.84	10.54
2010-02	71.93	3.4	8.3	13.1	0.8	1.19	8.03
2010-03	24.04	4.6	10.3	16	0	2.13	11.87
2010-04	83.81	4.1	10.5	16.9	1.9	1.36	12.28
2010-05	9.84	6.2	13.4	20.6	1.3	2.63	17.82

Appendix S6  
(continued)

Stark Creek

*C. unguiculata*

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	14.09	5.6	12	18.4	-2.8	3.6	16.16
2009-12	64.39	1.5	6.9	12.3	-3.1	1.73	8.82
2010-01	64	3.2	8.7	14.1	-1.3	1.84	10.54
2010-02	71.93	3.4	8.3	13.1	0.8	1.19	8.03
2010-03	24.04	4.6	10.3	16	0	2.13	11.87
2010-04	83.81	4.1	10.5	16.9	1.9	1.36	12.28
2010-05	9.84	6.2	13.4	20.6	1.3	2.63	17.82

*C. unguiculata* Live Oak

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	13.72	5.6	12	18.5	-2.5	3.53	16.09
2009-12	63.62	1.5	6.9	12.4	-3	1.72	8.86
2010-01	63.42	3.3	8.7	14.2	-1.3	1.84	10.65
2010-02	71.43	3.4	8.3	13.2	1.2	1.11	7.97
2010-03	23.36	4.6	10.4	16.1	0.6	1.95	11.68
2010-04	80.7	4.2	10.6	17	2.2	1.28	12.26
2010-05	9.23	6.3	13.5	20.7	1.5	2.57	17.92

## Appendix S6 (continued)

*C. unguiculata* Democrat  
Spgs  
Cmpgrnd

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	18.89	4.1	11.1	18.1	-5	3.51	16.37
2009-12	94.78	0.3	6.1	12	-4.5	1.73	9.12
2010-01	80.91	1.8	7.7	13.5	-2.5	1.65	10.41
2010-02	104.21	2.2	7.5	12.7	-0.9	1.28	8.44
2010-03	27.1	3.3	9.3	15.2	-2.3	2.32	11.79
2010-04	91.56	3.1	9.5	16	0.8	1.37	11.53
2010-05	13.27	5.4	12.6	19.7	0.9	2.31	16.63
2010-06	0.74	11.8	20.5	29.1	4.4	5.69	31.06

*C. unguiculata* China  
Gardens

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	18.93	3.8	10.9	18	-5	3.38	16.26
2009-12	92.76	0.1	6	12	-4.6	1.67	9.23
2010-01	79.97	1.6	7.4	13.3	-2.5	1.55	10.2
2010-02	101.7	2.1	7.4	12.8	-0.8	1.21	8.51
2010-03	27.01	3.1	9.1	15.2	-2.4	2.26	11.96
2010-04	87.8	3	9.5	16	0.7	1.32	11.67
2010-05	13.36	5.3	12.6	19.8	0.9	2.23	16.83
2010-06	0.74	11.6	20.4	29.2	4.5	5.43	31.25

Appendix S6  
(continued)

*C. xantiana*  
ssp.  
*parviflora*

Fay Ranch  
Road

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	12.46	3.8	11.2	18.7	-5	3.45	17.43
2009-12	81.21	0.5	6.4	12.3	-4.7	1.84	9.55
2010-01	94.32	1.6	7.6	13.6	-3.3	1.74	10.65
2010-02	90.16	2.4	7.9	13.4	-1.1	1.37	9.38
2010-03	34.73	3.6	10.1	16.6	-2.8	2.65	14.08
2010-04	50.73	3.4	10.5	17.6	-0.9	2.13	14.87
<b>2010-05</b>	<b>11.75</b>	<b>7.6</b>	<b>14.5</b>	<b>21.5</b>	<b>-0.2</b>	<b>4</b>	<b>20.18</b>

*C. xantiana*  
ssp.  
*parviflora*

Long Valley

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	9.87	4.6	9.3	14	-8	5.4	13.67
2009-12	63.45	-1.3	3.6	8.5	-8.7	2.22	7.8
2010-01	102.58	0.3	4.6	8.9	-6.8	2.43	8.22
2010-02	70.69	-0.2	4.4	9	-6.3	1.57	7.54
2010-03	22.99	0.4	6.2	11.9	-8.1	2.84	10.76
2010-04	31.13	0.9	7.5	14.2	-5.3	2.38	12.2
2010-05	0.4	3.5	10.8	18.1	-6	3.76	16.16
<b>2010-06</b>	<b>0</b>	<b>11.1</b>	<b>19.3</b>	<b>27.5</b>	<b>-0.6</b>	<b>7.74</b>	<b>28.91</b>

## Appendix S6 (continued)

*C. xantiana*  
ssp.  
*parviflora*Sawmill  
Road

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	15.68	3.3	11	18.8	-4.5	3.07	17.19
2009-12	80.06	-0.1	6.2	12.6	-4.1	1.5	9.52
2010-01	83.72	1.3	7.6	13.9	-2.2	1.43	10.52
2010-02	93.8	2.2	7.8	13.5	-0.6	1.13	9.09
2010-03	28.2	3.2	9.8	16.4	-1.6	2.07	12.98
2010-04	45.93	3.3	10.1	16.9	0.6	1.54	12.88
2010-05	14.46	6.5	13.7	20.8	0.9	2.93	18.43

*C. xantiana*  
ssp. *xantiana*Democrat  
Spgs  
Campground

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	18.89	4.1	11.1	18.1	-5	3.51	16.37
2009-12	94.78	0.3	6.1	12	-4.5	1.73	9.12
2010-01	80.91	1.8	7.7	13.5	-2.5	1.65	10.41
2010-02	104.21	2.2	7.5	12.7	-0.9	1.28	8.44
2010-03	27.1	3.3	9.3	15.2	-2.3	2.32	11.79
2010-04	91.56	3.1	9.5	16	0.8	1.37	11.53
2010-05	13.27	5.4	12.6	19.7	0.9	2.31	16.63
2010-06	0.74	11.8	20.5	29.1	4.4	5.69	31.06

## Appendix S6 (continued)

*C. xantiana*  
ssp. *xantiana* Borel Road

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	18.68	3	10.8	18.6	-4.6	2.87	16.97
2009-12	72.76	0	6.4	12.8	-3.9	1.45	9.78
2010-01	79.57	1.4	7.6	13.8	-1.8	1.31	10.24
2010-02	90.18	2.3	8	13.7	0.3	1.04	8.83
2010-03	27.69	3.2	9.6	16.1	-1.3	2.02	12.53
2010-04	49.18	3.5	10.2	16.9	1.2	1.38	12.49
2010-05	14.13	6.6	13.7	20.7	1.2	2.75	18.21

*C. xantiana*  
ssp. *xantiana* Camp 3

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	13.46	3.2	10.8	18.4	-4.5	3.06	16.83
2009-12	111.28	-0.2	5.9	12	-5.1	1.71	9.26
2010-01	98.49	1.1	7.2	13.2	-3.2	1.58	10.3
2010-02	105.89	1.8	7.3	12.8	-1.8	1.35	8.9
2010-03	33.39	2.6	9.2	15.8	-2.5	2.21	12.63
2010-04	58.67	2.6	9.7	16.7	0	1.56	12.78
2010-05	14.3	6	13.2	20.4	0.5	2.84	17.94

## Appendix S6 (continued)

*C. xantiana*  
ssp. *xantiana*      Sawmill  
Road

Date (year-month)	ppt (mm)	Temp, minimum (°C)	Temp, mean (°C)	Temp, max (°C)	Mean dew point temperature (°C)	VPDmin (hPa)	VPDmax (hPa)
2009-11	17.31	3.6	10.7	17.9	-5.2	3.47	16.38
2009-12	97.43	-0.3	5.5	11.4	-5.2	1.75	8.73
2010-01	91.59	1.1	7.2	13.2	-3.4	1.75	10.5
2010-02	108.51	1.1	6.6	12.2	-2.3	1.29	8.41
2010-03	29.65	2.5	8.7	14.8	-2.7	2.19	11.32
2010-04	58.22	2.2	8.7	15.3	0.1	1.24	10.78
2010-05	14.12	5.2	12.1	19	0.5	2.47	15.55
2010-06	0.03	11.9	20.2	28.5	4.2	5.99	29.45

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Appendix S7. Application of Bonferroni correction to the tests designed to detect significant differences between sister taxa (*Clarkia unguiculata* vs. *C. exilis* and *C. xantiana* ssp. *xantiana* vs. *C. xantiana* ssp. *parviflora*) with respect to physiological traits measured during vegetative growth and during flowering. All of the 12 tests (3 physiological traits x 2 life history stages x 2 pairs of sister taxa) that were statistically significant at  $\alpha = 0.05$  remained statistically significant at  $\alpha = 0.05/12 \approx 0.004$ . See Fig. 6 for comparison of means between sister taxa.

**Taxon effect (*unguiculata* - *exilis*), 99.6% CIs**

<b>Response Variable</b>	<b>Stage</b>	<b>Effect</b>	<b>CI_lower</b>	<b>CI_upper</b>	<b>significant</b>
Photosynthesis	Vegetative	-0.669	-3.394	2.210	FALSE
Photosynthesis	Flowering	-6.102	-8.999	-3.274	TRUE
Transpiration	Vegetative	0.001	-0.001	0.002	FALSE
Transpiration	Flowering	-0.005	-0.007	-0.003	TRUE
Water use efficiency	Vegetative	-0.017	-0.048	0.016	FALSE
Water use efficiency	Flowering	0.057	0.036	0.084	TRUE

**Taxon effect (*xantiana* - *parviflora*), 99.6% CIs**

<b>Response Variable</b>	<b>Stage</b>	<b>Effect</b>	<b>CI_lower</b>	<b>CI_upper</b>	<b>significant</b>
Photosynthesis	Vegetative	-1.242	-4.128	1.842	FALSE
Photosynthesis	Flowering	2.046	-1.504	5.898	FALSE
Transpiration	Vegetative	-0.005	-0.006	-0.003	TRUE
Transpiration	Flowering	0.001	-0.001	0.003	FALSE
Water use efficiency	Vegetative	0.046	0.031	0.064	TRUE
Water use efficiency	Flowering	-0.044	-0.112	0.030	FALSE

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Appendix S8. Cumulative precipitation and means of monthly climatic parameters from November 2007-June 2008 and November 2009-June 2010 at each of the 11 field sites where populations were surveyed in the current study. Values based on monthly values extracted from the PRISM data base, interpolated for each site within the 4 km resolution grid cells. Relative to 2007-08, the 2009-2010 growing season had higher cumulative precipitation, a lower mean monthly mean temperature and lower mean monthly minimum and maximum vapor pressure deficits.

Taxon/taxa present	Site	Growing season (Nov-June)	Nov-Jun Cumulative PPT (mm)	Nov-June Tmean (°C)	Nov-June VPDmin (hPa)	Nov-June VPDmax (hPa)
<i>C. exilis</i>	Willow Springs	2007-08	190.8	13.2	2.1	19.3
<i>C. exilis</i>	Willow Springs	2009-10	307.1	12.5	1.4	15.6
<i>C. exilis</i> and <i>C. unguiculata</i>	Stark Creek	2007-08	194.0	11.6	3.2	17.1
<i>C. exilis</i> and <i>C. unguiculata</i>	Stark Creek	2009-10	332.7	11.4	2.6	14.9
<i>C. unguiculata</i>	Live Oak	2007-08	189.0	11.7	3.2	17.1
<i>C. unguiculata</i>	Live Oak	2009-10	326.0	11.5	2.5	14.9
<i>C. unguiculata</i> and <i>C. xantiana</i> ssp. <i>xantiana</i>	Democrat Spgs Campground	2007-08	281.2	11.0	3.0	16.9
<i>C. unguiculata</i> and <i>C. xantiana</i> ssp. <i>xantiana</i>	Democrat Spgs Campground	2009-10	431.5	10.5	2.5	14.4
<i>C. unguiculata</i>	China Gardens	2007-08	278.0	11.0	3.0	17.1
<i>C. unguiculata</i>	China Gardens	2009-10	422.3	10.4	2.4	14.5
<i>C. xantiana</i> ssp. <i>xantiana</i>	Borel Road	2007-08	238.7	11.5	2.9	18.6

<i>C. xantiana</i> ssp. <i>xantiana</i>	Borel Road	2009-10	352.7	10.9	2.3	15.3
<i>C. xantiana</i> ssp. <i>xantiana</i>	Camp 3	2007-08	283.9	11.0	2.9	18.2
<i>C. xantiana</i> ssp. <i>xantiana</i>	Camp 3	2009-10	435.5	10.5	2.5	15.0
<i>C. xantiana</i> ssp. <i>xantiana</i>	Sawmill Road	2007-08	314.3	10.2	3.0	16.1
<i>C. xantiana</i> ssp. <i>xantiana</i>	Sawmill Road	2009-10	416.9	10.0	2.5	13.9
<i>C. xantiana</i> ssp. <i>parviflora</i>	Fay Ranch Road	2007-08	248.6	11.7	3.5	19.6
<i>C. xantiana</i> ssp. <i>parviflora</i>	Fay Ranch Road	2009-10	375.4	11.3	3.0	16.5
<i>C. xantiana</i> ssp. <i>parviflora</i>	Sawmill Road	2007-08	262.2	11.4	3.0	18.5
<i>C. xantiana</i> ssp. <i>parviflora</i>	Sawmill Road	2009-10	361.9	11.0	2.5	15.5
<i>C. xantiana</i> ssp. <i>parviflora</i>	Long Valley	2007-08	230.6	8.8	4.5	14.7
<i>C. xantiana</i> ssp. <i>parviflora</i>	Long Valley	2009-10	301.1	8.2	3.5	13.2
	<b>Cross-site Mean</b>	<b>2007-08</b>	<b>246.5</b>	<b>11.2</b>	<b>3.1</b>	<b>17.6</b>
	<b>Cross-site Mean</b>	<b>2009-10</b>	<b>369.4</b>	<b>10.7</b>	<b>2.5</b>	<b>14.9</b>